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VOL. 24 No. 1-2 2011

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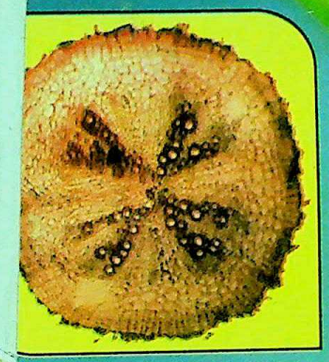

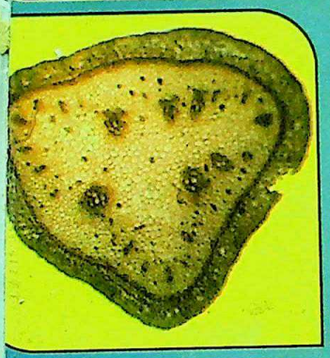

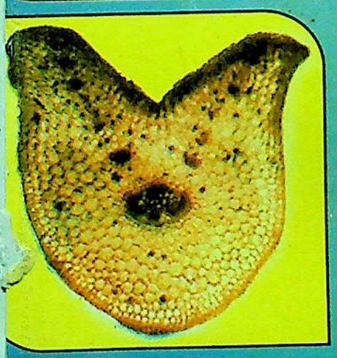

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* To provide a plateform to the Scientists, Professors, Teachers, Experts and Students related with plant sciences for exchange their views on nature and botanical structures and functions.
* To organzse Seminars, Conference, Workshops etc, for encouraging close interaction amongst Scientists, Research Scholars, Professors, Experts etc.
* To disseminate the knowledge of Plant Sciences for the improvement, conservation and management of plants, without which the life is not possible on earth.

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The membership of the Academy is open to all persons interested in Plant Sciences. For individual annual membership and Institution annual membership please contact to Secretary APSI. All members are entitled to obtain copies of the Journal gratis and all other publications of Academy at half price.

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A Report

0th APSI Scientists Meet & National Conference on Current Innovations in Plant Sciences (5-7 Feb. 2011) at Kopargaon, Maharashtra

Sponsored by University of Pune

131903

20th APSI Scientists Meet and National Conference on Current Innovations in ant Sciences sponsored by University of Pune and organized by Dept. of Botany, J. Somaiya College of Arts, Commerce & Science, Kopargaon, Dist. mednagar, a NAAC accredited 'B' Level College on 5th to 7th Feb. 2011. nference was inaugurated by Prof. Vidyavati, Former Vice-Chancellor, Kakatiya iversity, Warangal, A.P. . On this occasion Dr. S.K. Gupta, Secretary and Chief tor of Academy of Plant Sciences, India, Dr. (Smt.) P. Shrivastava from Dept. Botany, University of Rajasthan, Jaipur, Prof. D. Anusuya, Bangalore University, ngalore, Prof. S.S. Deokule, Head, Dept. of Botany, University of Pune, Dr. G. Bansode- Former Principal, Ahmednagar College, Ahmednagar, Hon. yaneshwar Waghchoure, Director, Ashwamedh Agri. and Farm Solution systems . Kopargaon, Hon. Sandip Rohamare, Member L.M.C., K.J. Somaiya College, pargaon, Hon. Ashokrao Rohamare, Chairman, K.T.E. Society, Kopargaon were sent.

Prof. Vidyavati delivered lecture of studies on biodiversity and sustainability Microalgae. Dr. Vidyavati express her views and gave best wishes for College gave emphasis on the women empowerment in Education and said Saraswati, shmi will naturally come, for the Conference how many womens are present is important but representative of women is there. Dr. Pushpa Shrivastava said in nature changes are continuously occurs if the changes are not accepted quickly it must be accepted lateron. She appreciate the encourage and upliftment of students by institute. Dr. S.S. Deokule said 'the rural institute like K.J. Somaiya ege of Arts, Commerce and Science arrange such type of National Conference he gave best wishes to Institute. Dr. S.D. P[illegible] of the College omed the delegates and highlight the major [illegible] ge especially education of girls at a cost of Rs. 10/- only.

131903

Hon. Chairman Ashokrao Rohamare, Chairman, K.T.E. Society said in his ch 'my father late K.B. Rohamare started this College in 1964 and later in Science faculty started on totally no grant basis, then became grantable. In three days discuss, interact and try to solve the problem of Global warming, tion, problems of bio-diversity.

Dr. S.K. Gupta told about Academy of Plant Sciences, impact factor of ational journal 'Advances in Plant Sciences'. For this Conference from Andhra esh, West Bengal, Rajasthan, U.P., Tamil Nadu, Assam, Karnataka and ent Universities of Maharashtra about 125 participants were present. Prof. D. uya from Bangalore and Dr. S.D. Pingle, Principal of K.J. Somaiya College, rgaon were honoured by APSI Gold medals.

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For first technical session Prof. P. Shrivastava worked as a chairperson where as Vipin Kumar from S.V.P. University of Agriculture and Technology Meerut (U.P.) worked as a rapporteur. In this session, plenary lecture was delivered by Dr. D. Anusuya on role Biofertilizer in organic farming. Then Dr. S.S. Deokule, Head, Dept. of Botany, University of Pune delivered lecture on 'Role of Ethnobotanical Research in Traditional Medicine and sustainable development'. Then delegates visited the medicinal plant nursery Ashwamedh Agri. and Farm Solution System Ltd., Kopargaon owned by director of K.T.E. Society, Dnyaneshwar Waghchoure, Entomologist, Winner of National Award for environmental Awareness.

On the second day of the conference in second technical session Dr.B.J. Apparao, Principal Jijamata College, Bhende, Dist. Ahmednagar delivered lecture on 'Genetic Improvement of Pulses through Induced mutations'. Then Dr. P. Shrivastava delivered lecture on ' Therapeutic Application of *Spirulina* in the treatment of rheumatoid Arthritis'. For this session Dr. A.G. Bansode former Principal worked as chairperson and Dr. Ravindra Kshirsagar Akurdi College, Pune worked as rapporteur.

In the afternoon session Dr. V.R. Gunale, Dept. of Botany, University of Pune and Ramshankar Basu, Principal, Saltora Netaji Centenary College, West Bengal worked as a experts for oral presentation and about 9 young scientist presented their work by oral presentation and about 8 scientist presented paper by poster presentation by this session. Dr. M.L. Ghosh, Hugali, West Bengal worked as a expert. Dr. V.R. Gunale from University of Pune delivered very informative lecture on 'How to use and calculate Dewates. At evening cultural programme for participants was organized by College and whole management and organization was done by Prof. S.K. Bansode, Dept. of English from our College and very nice programme was performed by girl students from the College, and programme of mouth organ was performed by Hon Kiran Parikh from Kopargaon. Prof. Bansode also performs the programme of very nice songs.

On third day in 4th technical session, Dr. M.L. Ghosh, Ex-Associate professor, Head, Dept. of Botany, Bejoy Narayan Mahavidyalaya, Hugali, West Bengal delivered lecture on 'Scientific Evaluation of medicinal and aromatic plants and their proper utilization and conservation for the welfare of rural people, uplifting rural economy in the Gaugatic Bengal', for this session. Dr. Jaganmohan Reddy worked as chairperson and Dr. Satish Verma worked as rapporteur. Then 3 young scientists presented their articles by oral presentation. For oral presentation Dr. M.L. Ghosh worked as chairperson where as Dr. Jaganmohan Reddy as rapporteur.

On the occussion of valedictory function Dr. M.L. Ghosh, West Bengal, Dr. S.K. Gupta, Secretary and chief editor, Academy of Plant Sciences, Muzaffarnagar (U.P.), Dr. Jaganmohan Reddy, Warangal (A.P.), Dr. Salgare, Mumbai were present.

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On this occasion Dr. Salgare express best wishes to institute. He said colleges and Universities arranges such conferences in five star hotels, but in this institute conference is arranged in beautiful premises. If anybody has knowledge expresses this knowledge with respect to each other. Dr. Jaganmohan Reddy said this conference will percolate the knowledge for students, scientists and farmers. Dr. M.L. Ghosh, the Chief guest of this function express his view regarding the global warming and loss of biodiversity, he said such conferences will focus on biodiversity conservation.

Hon. Ashokrao Rohamare, President of Valedictory function and chairman of Kopargaon Taluka Education Society express his view, he said,' this conference for three days will be beneficial to the students, farmers and common peoples from society. Many social issues will be solved by this conference; representatives from participants also express their views.

On this occasion local management committee member Hon. Sandiprao Rohamare, L.M.C. member of College, was also present. Principal and Organizing Secretary presented the report of three days conference, Prof. S.K. Bansode and Prof. S.R. Pagare anchored the function and Prof. B.S. Gaikward introduce the chief guests and Dr. G.S. Shinde express vote of thanks.

In this conference Dr. R.R. Sanap (Kopargaon), Dr. A.B. Gore (Shrigonda), Dr. S.D. Pingle (Kopargaon), Dr. Ramshankar Basu (West Bengal), Miss. Prajakta Maygaonkar (Mumbai), Sarang Bokil (Pune) were awarded medal by Dr. S.K.Gupta Muzaffarnagar (U.P.) Chief editor and Secretary Academy of Plant Sciences. Dr.S.K. Gupta on behalf of Academy of Plant Sciences honoured Principal Dr. S.D. Pingle and College President Hon. Ashokrao Rohamare by presenting them Shawl and honoured Smt. Pingle by presenting Momento for organizing the conference is such a nice way and congratulate them for the success of conference.

Excellent arangement were made by the organizers for comfortable stay of delegates in the Dwaravati Bhakat Niwas at **Sirdi**. Delegates visited the temple of Sirdi Sai Baba and get blessed by Darsan of **Sai Baba**. Intrested delegate also went for Darsan of **Shani Mandir** at **Singnapur**, the only village of India where there are no doors in the houses. Some scientists also went to visit Nashik and Aurangabad.

Compiled by

Dr. S. K. Gupta
Secretary Academy of Plant Sciences India
Muzaffarnagar, (U.P.)

Dr. B.S. Gaikward
Dept. of Botany
K.J. Somaiya College
Kopargaon (M.S.)

Dr. S.D. Pingle
Principal
K.J. Somaiya College
Kopargaon (M.S.)

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ERRATUM

Correction of first author's name in the paper on Page No. 43.

The article named "**Studies on the Antibacterial, Antifungal and Antioxidant activities of *Saussurea costus* - A critically endangered medicinal plant of N.W. Himalaya.**" print in june, 2011 edition. The first author's name has been wrongly written as **G. Gazanfar** instead of **K.G. Mustafa**. Kindly read **K.G. Mustafa** instead of **G. Gazanfar** on Page No. 43 of Vol. 24 No. I (June 2011 issue) of Advances in Plant Sciences.

About APSI Scientist

Dr. S.P. Giri, Dept. of Botany, Arts, Commerce and Science College Satral, Maharashtra, has been awarded the degree of Doctor of Philosphy by University of Pune, wide his noticification No. PGS/ Ph.D./172 dated 14th March 2011. He worked on topic "Mutation Breeding of Pigeonpea [*Cajanus cajan* (L.) Mill sp.] for Yield Contributing Traits." under the Guidance of Dr. B.J. Apparao, Principal, Jijamata College of Science and Arts, Bhende Bk. Tal. Newasa Dist. Ahmednagar (MS).

APSI Awards

ABOUT APSI AWARDS

APSI

The International Medals and Citation are awarded annually by APSI in recognisation of sustained, outstanding contribution to the subject Plant Science. It is open to candidates of any nationality, subject to nomination, it is expected that those winning the awards should have published as significant proportion of their findings in this journal. For any other information, please write to Secretary, APSI 770, South Civil Lines, Muzaffarnagar-251001, India.

1. Young Scientist Award
2. Distinguished Indian Plant Scientist Award
3. APSI Honours Award
4. Salgare National Award. (Sponsored by Dr. S.A. Salgare, Mumbai)
5. Smt. Kamla Devi and Shri Ram Lal Dhiman APSI Award (Sponsored by Dr. A.K. Dhiman, Haridwar)
6. APSI International Award (Sponsored by M/s Ankur Seeds Pvt. Ltd., Nagpur)
7. (Rev.) Smt. Rama Devi APSI Award (Sponsored by Professor Ganesh Kumar, Pantnagar, Uttarakhand)

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Note :- If you wish to sponsore a Award - Please Contact.

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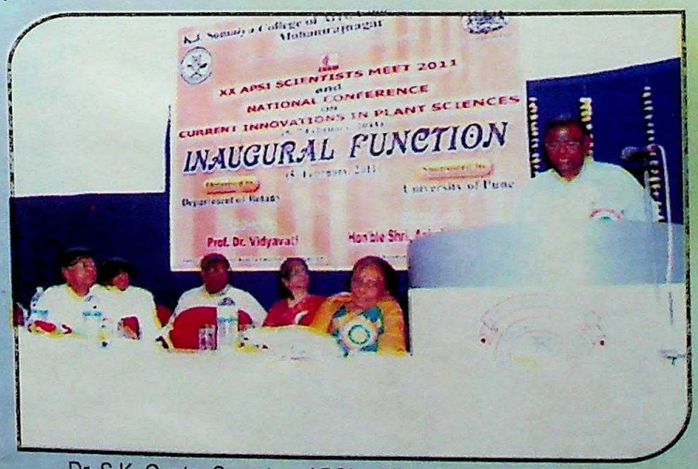
Photographs of 20th APSI Scientist Meet 2011 and National Conference at Kopargaon (Maharashtra) on 5th, 6th & 7th Feb. 2011

A view of Dias at Inaugration L.R. - Phof. P. Srivastava, Jaipur, Hon. Ashokrao Rohamare Chairman K.T.E. Society, Prof. S.S. Deokule, Pune, Dr. S.K. Gutpa, Secretary APSI, Prof. D. Anusuya (Bangalore), Dr. A.G. Bansode (Ahmednagar), Prof. Vidyavati Former Vice-Chancellor of Kakatiya University, Warangal, delivering the inaugral address.

A view of Dias Releasing the Souvenir.
L-R : Dr. S.A. Salgare, Prof. P.Srivastava, Hon. Ashokrao, Dr. S.K. Gupta, Prof. Vidyavati

Another View of Dias, Prof. Pushpa Srivastava of Rajasthan University, Jaipur Addressing the Audience

Dr. S.S. Deokule, Head, Dept. of Botany University of Pune, Addressing the Conference



Dr. S.K. Gupta, Secretary APSI, welcoming the delegates and presenting the thanks to Dr.S.D. Pingle, Principal, College management and staff for organizing the conference

Prof. D. Anusuya and Dr. S.D. Pingle recieptent of APSI Gold Medal and Citation with Chief guest and others.

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Photographs of 20th APSI Scientist Meet 2011 and National Conference at Kopargaon (Maharashtra) on 5th, 6th & 7th Feb. 2011

A View of Delegate in the Conference
In front line L-R : Dr. R.S. Basu from West Bengal, Dr. Vipin Kumar from Meerut

Another View of Delegate in the Conference

Dr. B.J. Apparao, Principal, Jijamata College, Bhende (MS) delivering Plenary lecture

Hon. Dnyaneshwar Waghchoure, Director, K.T.E. Society Kopargaon Addressing the Audience

Another view of delegate in conference during technical session
In front line - L to R, Dr. S.K. Gupta, Smt. Kusum Lata
Prof. D. Anusuya, Prof. Vidyavati

Dr. M.L. Ghosh from West Bengal addressing the delegates in Valedictory Function

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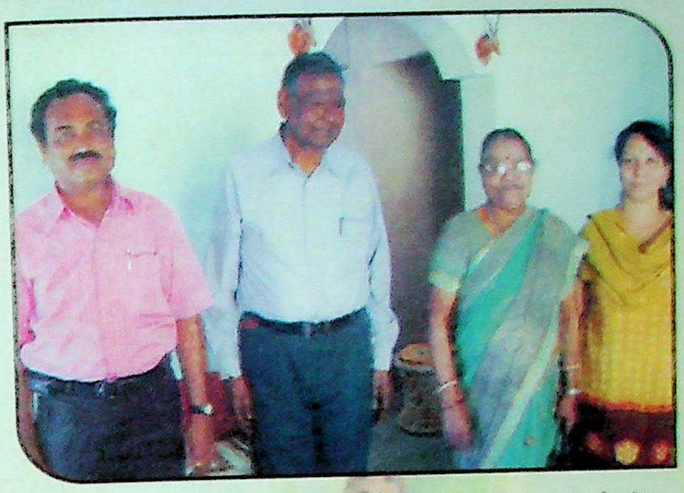
Photographs of 20th APSI Scientist Meet 2011 and National Conference at Kopargaon (Maharashtra) on 5th, 6th & 7th Feb. 2011

Dr. R.S. Basu, Principal, Saltora (W.B.) receiving Gold Medal at Kopargaon (M.S.) from Dr. S.K. Gupta, Secretary APSI, Dr. S.A. Salgare from Mumbai is also with them.



Dr. R.R. Sanap, Dr. S.K. Gupta, Smt. Kusum Lata and Smt. Sanap at Kopargaon (M.S.)

Smt. Sanap Honouring Smt. Kusum Lata at Kopargaon

Dr. R.R. Sanap presenting Shawl and Nariyal to Dr. S.K. Gupta at Kopargaon (M.S.)

Dr. A.B. Gore of Shrigondha (M.S.), Dr. S.K. Gupta, Dr. K.M. Patel (Mehsana), Mr. Rajesh (MZN), Dr. Devi Dayal (Bhuj, Gujarat), Dr. K. Kadavul (Pondicherry) and some lady delegates at Hyderabad Conferene.

Dr. V.D. Mendhulkar (Mumbai), Dr. S.K. Gupta (Muzaffarnagar), Professor P. Kaushik (Hardwar) and Dr. R.L. Ram (Ranchi) and others attending the conference at Hyderabad.

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SOME NEWS Regarding 20th APSI Scientist Meet 2011 and National Conference at Kopargaon (Maharashtra) on 5th, 6th & 7th Feb. 2011

दैनिक लोकमत
गुरुवार 10 फेब्रुवारी, 2011

संशोधनाकडे लक्ष केंद्रीत करावे- विद्यावती

अखिल भारतीय शास्त्रज्ञ परिषदेचा समारोप

कोपरगाव, दि.९(प्रतिनिधी)- सरस्वतीला नमस्कार केला की, लक्ष्मी आपोआप मिळते. त्यामुळे शिक्षक, शास्त्रज्ञ व विद्यार्थ्यांनी ज्ञाननिष्ठेच्या भूमिकेतून संशोधनाकडे लक्ष केंद्रीत करावे, असे आवाहन काकतिय विद्यापीठाच्या कुलगुरू डॉ. विद्यावती यांनी केले.

पुणे विद्यापीठ व येवला के.जे. सोमैया महाविद्यालयाच्या संयुक्त विद्यमाने विद्यार्थी अखिल भारतीय शास्त्रज्ञ परिषद व 'करंट इन्डेक्शन इन प्लांट सायन्सेस' या विषयावरील राष्ट्रीय परिषदेच्या उद्घाटन प्रसंगी प्रमुख पाहुणे म्हणून या बोलत होत्या. अध्यक्षस्थानी संस्थेचे अध्यक्ष अशोकराव रोहमारे तर डॉ. एस.के. गुप्ता, प्रा. पुष्पा श्रीवास्तव, डॉ. अनुसया, प्रा. एस.एस. देवकुळे प्रमुख व्यासपीठावर उपस्थित होते.

प्रारंभी डॉ. एस.डी. पिंगळे यांनी प्रास्ताविकात परिषदेच्या आयोजनामागची भूमिका विशद केली. परिषदेसाठी विविध सत्रांतून १२५ विद्यार्थी व शास्त्रज्ञ उपस्थित होते. पुणे विद्यापीठाच्या वनस्पतीशास्त्र विभागाचे प्रमुख देवकुळे यांनी पारंपरिक औषधे व नित्य विकास या विषयावर, बंगलोर विद्यापीठाच्या डॉ. डी. अनुसूया यांनी सेंद्रीय शेतीत जैविक खताचे महत्व या विषयावर मार्गदर्शन केले. कोपरगाव येथील ज्ञानेश्वर जाकसे 'मांच्या आजारांवर औषधी वनस्पती' या विषयावर विद्यार्थ्यांशी संवाद साधला. प्राचार्य डॉ. एस.डी. पिंगळे यांनी उपस्थितांचे आभार मानले.

पुण्य नगरी
मासिक - अहमदनगर • वर्ष ११ • अंक ३०९ • शनिवार, १२ फेब्रुवारी २०११ • पाने १२ • किंमत ₹ २.००

अन्नाची कमतरता दूर करण्यासाठी वनस्पती अभ्यासकांनी झटावे -डॉ. विद्यावती

कोपरगाव /प्रतिनिधी

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सकाळ

ज्ञाननिष्ठेच्या भूमिकेतून संशोधनाकडे लक्ष द्यावे

प्रा. विद्यावती यांचे प्रतिपादन; कोपरगावमध्ये राष्ट्रीय परिषद

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२ लोकसत्ता
रविवार, १३ फेब्रुवारी २०११

वृत्तान्त

'शिक्षक, शास्त्रज्ञ व विद्यार्थ्यांनी ज्ञाननिष्ठेच्या भूमिकेतून संशोधनाकडे लक्ष केंद्रित करावे'

कोपरगाव, १२ फेब्रुवारी/वार्ताहर

सरस्वतीला नमस्कार केला की लक्ष्मी आपोआपच मिळते. त्यामुळे शिक्षक, शास्त्रज्ञ व विद्यार्थ्यांनी ज्ञाननिष्ठेच्या भूमिकेतून संशोधनाकडे लक्ष केंद्रीत करावे, असे आवाहन काकतिय विद्यापीठाच्या माजी कुलगुरू डॉ. विद्यावती यांनी केले.

पुणे विद्यापीठ व के. जे. सोमय्या महाविद्यालयाच्या वतीने अ. भा. शास्त्रज्ञ परिषद व 'करंट इन्डेक्शन इन प्लॅन्ट सायन्सेस' या विषयावरील राष्ट्रीय परिषदेच्या उद्घाटनप्रसंगी त्या बोलत होत्या. अध्यक्षस्थानी संस्थेचे अध्यक्ष अशोकराव रोहमारे होते. डॉ. एस. के. गुप्ता, प्रा. पुष्पा श्रीवास्तव, डॉ. डी. डी. अनुसया, प्रा. एस. एस. देवकुळे यावेळी उपस्थित होते. डॉ. विद्यावती म्हणाल्या की, मी मागील ५० वर्षांपासून मानसशास्त्राचा अभ्यास करते आहे. तो करताना संशोधनात निष्कर्ष लवकर काढता येत नाही असा अनुभव आला. त्यामुळे विद्यार्थ्यांनी संशोधनासाठी चिकाटी व कठोर परिश्रमाची तयारी ठेवावी.

यावेळी बोलताना प्रा. श्रीवास्तव म्हणाल्या की, देशाची वाढती लोकसंख्या लक्षात घेता या दशकातच अन्नाची कमतरता जाणवणार आहे. ती भरून काढण्याचे कार्य आपण सर्व वनस्पती शास्त्रज्ञांना व अभ्यासकांना करायचे आहे. श्री. रोहमारे म्हणाले की, परिषदेत सर्व शास्त्रज्ञांनी एकत्र येऊन पर्यावरण, जैवविविधता व जागतिक सापमान वाढ या घटकांवर चर्चा करून चांगला निष्कर्ष काढा. प्राचार्य डॉ. एस. डी. पिंगळे यांनी प्रास्ताविक केले.

परिषदेत आंध्र प्रदेश, पश्चिम बंगाल, राजस्थान, उत्तर प्रदेश, महाराष्ट्र, तामिळनाडू, आसाम, कर्नाटक या राज्यांतील १२५ विद्यार्थी व शास्त्रज्ञ सहभागी झाले होते.

डॉ. देवकुळे यांनी पारंपरिक औषधे व निरंतर विकास, डॉ. अनुसया यांनी सेंद्रीय शेतीत जैविक खताचे महत्त्व, बी. जे. अप्पाराव यांनी जनुकीय बदलाने पीक उत्पादन, डॉ. श्रीवास्तव यांनी शेवाळाच्या संवेदुखोवर वापर या विषयांवर मार्गदर्शन केले.

परिषदेच्या समारोपप्रसंगी सलगरे यांचे भाषण झाले. डॉ. जगमोहन रेड्डी म्हणाले की, तरुण शास्त्रज्ञ जे काम करतात ते निश्चितच विद्यार्थी, शास्त्रज्ञ व शेतकरी यांना मार्गदर्शक ठरेल. एम. एल. घोष यांचे भाषण झाले. यावेळी संदीप रोहमारे, अनिल सोमवंशी उपस्थित होते. प्रा. जी. एस. शिंदे यांनी आभार मानले. सूत्रसंचालन प्रा. एस. के. बनसोडे व प्रा. एस. आर. पगारे यांनी केले.

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Correction shoulde be - Department of Biotechnology, National institute of Technology, Durgapur (W.B.) on Page No. 439

So, Please read West Bengal (W.B.) in place of Chhattishgarh (C.G.) on Page 439

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In Vitro Plant Regeneration from Leaf and Stem Explants of *Solanum xanthocarpum* Schrad & Wendl. - An Important Medicinal Herb

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Induction of callus and multiple shoots using leaf and stem explants of *Solanum xanthocarpum* was achieved. Leaf and stem explants were cultured on MS (Murashige and Skoog) medium supplemented with different concentrations of IAA, NAA, BAP and Kn alone. The frequency of callus induction increased with increasing concentrations of all growth regulators used. The optimal levels were formed to be 11.4 μML^{-1} of IAA / 10.7 μML^{-1} of NAA and 8.8 μML^{-1} BAP / 9.3 μML^{-1} Kn. Among the different growth regulators tried, auxins IAA and NAA alone induced whitish and brownish friable callus, where as BAP and Kn induced greenish friable and green compact callus. Leaf was found to be more efficient in producing callus and multiple shoots. Shoots were induced from green compact callus cultures of these explants on the same medium. Maximum number of multiple shoots was obtained from leaf derived callus. The regenerated shoots were rooted on MS medium without growth regulators, and were successfully hardened and transferred to the field.

Keywords : *Solanum xanthocarpum,* Leaf, Stem, Callus, Regeneration.

INTRODUCTION

Solanum xanthocarpum Schrad & Wendl. is a medicinally important member of Solanaceae. All the parts of *S. xanthocarpum* were used as traditional Ayurvedic medicinal herb in India. It was used for TB, fever, asthma, lung diseases and kidney disorders. Pharmacological studies on this herb have shown that aqueous and alcoholic extracts of the plant possess hypotensive effect, which is partly inhibited by atropine. The species contains steroidal alkaloids, solasonine and solamargine. Solasodine serves as an important intermediate in synthesis of steroidal hormones (Butcher, 1977) and is a potential alternative to diosgenin as a precursor in synthesis of steroidal hormones (Macek, 1989). Most of the medicinal plants are even today collected from the wild sources. The pressure on the natural population of medicinal herbs is so serious that several precious medicinal plants are likely to be designated as threatened, vulnerable, endangered, extinct etc. Tissue culture techniques are also being developed for the mass propagation of medicinal plants are of prime importance for mass production and conservation of the particular species or endangered species because of their medicinal properties. There is a need to employ biotechnological methods to improve the yield of metabolites for their medicinal purposes.

Only limited success has been reported for *in vitro* organogenesis and regeneration in *Solanum* species (Bhatt ans Sussex 1979; Macek 1989; Baburaj and Thamizhchelan, 1991; Bhalsingh and Maheswari, 1997).

Present communication reports a method of multiple shoots induction from leaf and stem explants of *S. xanthocarpum.*

MATERIAL AND METHODS

Leaf and stem explants were collected from the college herbal garden. The explants were washed with running tap water for 30 minutes and surface sterilized, initially in 70% ethanol for 2 min, followed by 0.1% (w/v) $HgCl_2$ for 1-2 min. and finally rinsed in sterile distilled water for 2-3 times. MS medium (Murashige & Skoog, 1962) with 3% sucrose (w/v) adjusted to pH 5.7 and solidified with 0.8% agar (w/v) and autoclaved at 1.05 Kg cm^{-2}, was used as a culture medium.

For organogenesis leaf and stem explants were cultured on MS medium fortified with various concentrations of IAA, NAA, BAP and Kn. All the cultures were maintained under white fluorescent light (80 $\mu EM^{-2}S^{-1}$) at the temperature 25 ± 2°C with 16hrs photoperiod.

The *in vitro* regenerated shoots (5 - 7 cm long) were transferred to MS medium for rooting. The *in vitro* regenerated plants were transferred to plastic cups containing the sterile soil mix red soil : sand : compost (1:1:1) and kept in green

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house. At least 20-24 explants were cultured in each treatment, and all the experiments were repeated three times.

RESULTS AND DISCUSSION

Callus induction : *S. xanthocarpum* leaf and stem explants were cultured on MS medium supplemented with different concentrations of IAA (0.6 - 28.5 μM), NAA (0.5 - 26.8 μM), BAP (0.4 - 22.2 μM) and Kn (0.5 - 23.2 μM) individually. Both explants were enlarged in size and thickened within a week time. Callus was initiated from surface of the explants after one week. IAA and NAA containing MS medium induced whitish and brownish friable callus, but BAP and Kn induced greenish friable and greenish compact callus (Table 1). Significant callus formation was observed within 10-15 days at the cut ends of the explants. Higher frequency of callus induction was achieved from leaf explants followed by stem explants. Compact green organogenic callus was observed in both explants in cytokinin supplemented medium (Fig 1 a & b). Among the different concentrations of auxins used for callus induction, 11.4 μML^{-1} IAA and 10.7 μML^{-1} NAA were found to be optimum, while increasing the auxin concentrations above this level reduced callus proliferation, Among the different concentrations of cytokinins used for callus induction, 8.8 μML^{-1} BAP and 9.3 μML^{-1} Kn were found to be optimum level for induction of organogenic calli. Baburaj and Thamizhchelvan (1991) observed similar results in *S. surattense*. Muthukumar and Arockiasamy (1998) reported that the 2.0mg/l BAP induced high amount of callus in *Datura metal*. Pawar *et al.*, (2002) observed that BAP and Kn were more effective for induction of organogenic callus from leaf explants of *S. surattense*. Thus cytokinins were suitable for induction of organogenic callus compared to auxin used in both explants *viz.*, leaf and stem as reported in literature.

Multiple shoots regeneration : Multiple shoots regeneration occurred through callus from both leaf and stem explants on the same fresh medium within 20-25 days. BAP (8.8 μM) and Kn (9.3 μM) produced highest shoot regeneration frequency as that of callus induction (Table 2). In the case of BAP leaf callus showed more regeneration capacity (77.6%) than stem callus. But in case of KN, we have observed opposite results, *i.e.*, stem callus produced the highest regeneration frequency (79%). Maximum number of shootlets developed from leaf explants at 8.8 μML^{-1} BAP whereas stem explants at 9.3 μML^{-1} Kn (Fig 1 c & d). Similar results were also observed in *S. surattense* by Pawar *et al.*, (2002) and Baburaj and Thamizhchelven (1991). Muthukumar and Arockiasamy (1998) reported that the BAP and Kn were more effective for induction of multiple shoots from leaf explants of *Datura metal*.

Root induction : Well-developed shoots were cultured on MS medium without growth regulators for *in vitro* rooting. Absolute percentage of shoots from both leaf and stem callus developed roots within a week (Fig 1 e). The rooted plantlets were transferred to plastic cups and kept in green house for 8-10 days, and then the plantlets were transferred to the field. Similar results were also reported by Pawar *et al.*, (2002) in *S. surattense*.

Table 1 : Effect of different concentrations of IAA, NAA, BAP & Kn on callus induction form leaf and stem explants of *S.xanthocarpum*.

Plant growth regulators (μML^{-1})	Leaf	Stem
	% of callus proliferation	% of callus proliferation
IAA		
0.6	22.3 ± 2.5^{v}	21.0 ± 1.7^{w}
2.8	37.6 ± 2.0^{r}	37.0 ± 2.0^{w}
5.7	61.0 ± 3.6^{j}	59.6 ± 3.5^{i}
11.4	80.0 ± 2.0^{c}	79.0 ± 1.0^{b}
17.3	72.3 ± 2.5^{e}	69.0 ± 1.0^{d}
22.8	60.0 ± 5.0^{k}	57.6 ± 2.5^{k}
28.5	46.6 ± 6.1^{o}	45.0 ± 5.0^{o}
NAA		
0.5	22.3 ± 2.0^{v}	21.6 ± 1.5^{w}
2.7	35.3 ± 3.5^{s}	32.3 ± 2.5^{t}
5.4	60.0 ± 2.6^{k}	58.0 ± 3.6^{k}
10.7	78.3 ± 2.0^{d}	76.3 ± 2.3^{c}
16.1	70.3 ± 1.5^{f}	67.3 ± 2.3^{e}
21.5	57.3 ± 3.0^{l}	55.0 ± 3.0^{l}
26.8	45.0 ± 5.0^{p}	44.3 ± 3.2^{p}
BAP		
0.4	29.6 ± 2.0^{t}	27.0 ± 2.6^{uv}
2.2	40.0 ± 2.0^{q}	38.0 ± 2.6^{r}
4.4	62.5 ± 2.5^{i}	61.3 ± 1.5^{h}
8.8	36.3 ± 2.5^{a}	82.6 ± 2.6^{e}
13.3	70.3 ± 1.5^{f}	68.0 ± 2.6^{e}
17.7	64.3 ± 2.5^{h}	59.0 ± 1.0^{j}
22.2	52.0 ± 2.0^{m}	49.0 ± 1.0^{n}
Kn		
0.5	27.3 ± 3.2^{u}	27.6 ± 3.0^{u}
2.3	38.3 ± 2.0^{r}	39.3 ± 2.0^{q}
4.6	61.0 ± 2.6^{i}	62.3 ± 2.5^{g}
9.3	82.0 ± 2.0^{b}	83.6 ± 2.5^{a}
13.9	67.3 ± 2.0^{g}	69.0 ± 1.7^{d}
18.6	59.6 ± 2.0^{k}	61.3 ± 2.5^{h}
23.2	50.0 ± 1.0^{n}	52.6 ± 3.2^{m}

Each value represents the mean ± SD of 20 replicates and each experiment was repeated thrice. Values with the same superscript are not significantly different at the 0.05% probability level according to DMRT.

Thus, this protocol is useful for conservation of this important medicinal plant and also for large-scale propagation.

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Table 2 : Effect of different concentrations of BAP and Kn on multiple shoots induction form leaf and stem callus of *S. xanthocarpum*

Plant growth regulators (μ ML^{-1})	Leaf			Stem		
	Regeneration (%)	Average no. of Shoot /explant ± SE	Average Shoot length (cm) ± SE	Regeneration (%)	Average no. of Shoot/ explant ± SE	Average Shoot length (cm) ± SE
BAP						
0.4	22.0 ± 2.0^{l}	12.3 ± 2.5^{i}	2.0 ± 1.0^{j}	20.0 ± 1.0^{k}	11.0 ± 1.0^{i}	1.0 ± 1.0^{h}
2.2	42.3 ± 2.5^{j}	19.0 ± 1.0^{f}	4.0 ± 1.0^{g}	40.6 ± 2.0^{i}	15.6 ± 2.0^{g}	3.3 ± 0.5^{f}
4.4	62.3 ± 2.5^{e}	22.6 ± 2.0^{d}	7.3 ± 1.5^{c}	60.3 ± 2.5^{e}	20.3 ± 1.5^{f}	6.6 ± 0.5^{c}
8.8	77.6 ± 2.5^{a}	32.0 ± 2.0^{a}	10.0 ± 1.0^{a}	75.0 ± 3.0^{b}	29.6 ± 1.5^{b}	9.6 ± 0.5^{a}
13.3	67.6 ± 2.5^{c}	24.0 ± 2.0^{c}	7.0 ± 1.0^{c}	61.3 ± 2.5^{d}	21.3 ± 1.5^{e}	6.3 ± 1.1^{c}
17.7	56.6 ± 2.0^{g}	17.0 ± 1.0^{g}	5.6 ± 1.1^{c}	52.3 ± 3.2^{g}	13.6 ± 1.5^{h}	5.0 ± 1.7^{d}
22.2	46.3 ± 1.5^{i}	12.0 ± 1.0^{i}	4.3 ± 1.5^{g}	41.0 ± 1.0^{i}	9.6 ± 1.5^{j}	3.6 ± 2.0^{e}
Kn						
0.5	20.6 ± 2.0^{m}	12.0 ± 2.0^{i}	1.6 ± 0.5^{j}	23.0 ± 1.0^{j}	14.0 ± 2.0^{h}	2.0 ± 1.0^{g}
2.3	41.3 ± 1.5^{k}	18.6 ± 0.5^{f}	3.3 ± 0.5^{j}	45.0 ± 3.0^{h}	21.0 ± 1.0^{e}	4.3 ± 0.5^{e}
4.6	61.3 ± 1.5^{f}	20.6 ± 2.0^{e}	6.6 ± 0.5^{d}	67.3 ± 2.5^{c}	22.0 ± 2.0^{d}	7.0 ± 1.0^{b}
9.3	76.0 ± 2.6^{b}	30.0 ± 1.0^{b}	8.3 ± 0.5^{b}	79.0 ± 1.0^{a}	33.3 ± 2.0^{a}	10.0 ± 1.0^{a}
13.9	65.0 ± 3.0^{d}	22.6 ± 2.5^{d}	6.6 ± 0.5^{d}	67.3 ± 2.5^{c}	23.0 ± 2.0^{c}	7.3 ± 1.1^{b}
18.6	52.0 ± 2.0^{h}	15.3 ± 2.5^{h}	5.3 ± 0.5^{f}	57.0 ± 2.6^{f}	16.6 ± 1.5^{g}	6.0 ± 1.0^{c}
23.2	42.6 ± 2.0^{j}	9.0 ± 1.0^{j}	3.6 ± 1.5^{a}	44.6 ± 4.1^{h}	10.3 ± 1.5^{j}	5.3 ± 0.5^{d}

Each value represents the mean ± SD of 20 replicates and each experiment was repeated thrice. Values with the same superscript are not significantly different at the 0.05% probability level according to DMRT.

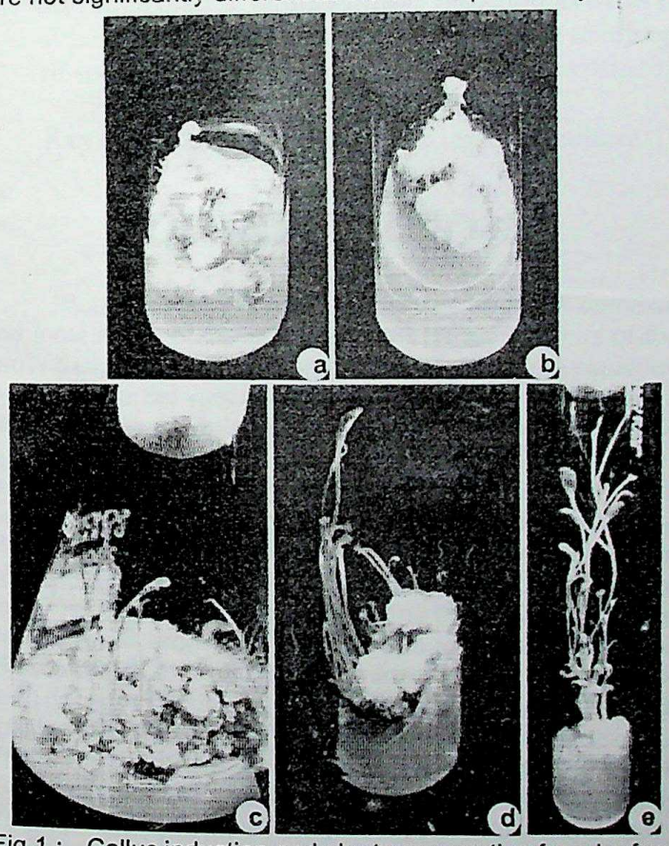

Fig 1 : Callus induction and plant regeneration from leaf and stem explants of *Solanum xanthocarpum*

(a) & (b) Callus induction from leaf and stem explants of MS + BAP 8.8 μML^{-1}

(c) & (d) Multiple shoot regeneration on MS + BAP 8.8 μML^{-1} and MS + Kn 9.3 μML^{-1} leaf and stem callus respectively

(e) Root induction on MS without growth regulators

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Embryo Culture and Seedling Invigoration for Better Conservation of Important Mangrove - *Ceriops*

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The fruits of *Ceriops* were collected from Sunderban areas of West Bengal at different days of pollination and it was found that after 15 days of pollination the fruits were at right stage for embryo extraction. At that stage the average fruit size was 23 ± 01 mm and extracted embryo and size was only 15 ± 0.3 mm. The seed moisture content was measured to be 65 ± 0.23 per cent indicating recalcitrant nature of seed. The average weight of the fruit was 850.00 ± 0.11 mg, whereas the extracted embryo weighed only 120.00 ± 0.21 mg, which indicates that the embryo was only 14.12 per cent of the fruit. In *Ceriops*, seed viability due to high salinity and pollution in natural habit at Sunderban, were the other factors which reduced the per cent of seedling survibility of *Ceriops* and was found to be only 61.00 ± 0.13 per cent. The embryo culture specially in MS-VI medium with BAP @ 1ml/l gave best results for increased embryo survivility upto 94.00 ± 1.02 per cent. Those invigorated seedlings were planted and the per cent of seedlings survived after embryo culture and invigoration treatment was recorded to be as high as 89 ± 0.74 per cent.
It has been concluded that the problem of seedling mortality at natural habitat can be reduced and per cent of seedling survival per unit area can be increased, helping in mangrove conservation and management.

Keywords : Mangrove, *Ceriops*, embryo culture.

INTRODUCTION

Sunderbans, the name is derived from the common and local name of the plant, Sundari *Heritiera fomes* of the family Sterculiacea. This intertidal deltic forest in the estuarine mouths of the two vast rivers the Ganga and the Brahmaputra has formed the single largest deltaic land. Here a diverse mangrove flora and vast group of terrestrial, avian and aquatic fauna are present. During partition of Bengal in 1947 more than 66% of the total Mangrove areas of Sunderbans had fallen within the jurisdictiion of Bangladesh and only 34% Mangrove forest remained within India. While about 500,000 hectares of Mangrove forest existed in India during the sixty's, but during eighty's, such area was brought down to, 200,000 hectares (Blasco, 1977). But subsequently, about 50% of this area has been depleted by 1986 (Untwale, 1986). At the same time, the species diversity has also decreased from 58 to 48 (Untawale, 1986). The Mangrove ecosystem, which is considered as one of the most productive ecosystem, is going to be ecologically unbalanced and the main causes of such depletion are mainly due to human interference, increased salinity and pollution of both soil and water by unplanned industrialization, causing increased loss of seed viability leading to less seedling survival percentage per unit area. Several tissue culture techniques have been developed for *Phalaenopsis*, orchids inculding the culture of flower stalks with axillary buds meristem, internodal segments of flower stalks (Arditti and Earnest, 1993; Park *et al.*, 1996; Poseas *et al.*, 2008). Among these techniques, the flower stalk culture is an excellent method for rapid clonal propagation (Tanaka, 1992). It is in this context the present investigation was undertaken to produce more viable embryo through embryo culture and invigorate those cultured embryo by standard Hoagland's nutritive solution for a certain period and then those invigorated seedlings were rehabilitated in their natural habitat, in Sundarbans for better establishment at such a polluted, substandard locality. By this technology, better conservation and management of this mangrove can be achieved.

MATERIAL AND METHODS

Material : *Ceriops decandra.*

Methods : Scalpels, needles, forceps etc. were sterilised and dipped in 70% alcohol and then flamed. The selected fruit surface was sterilized by 0.1% Bavistin (fungicide) and 0.1% Mercuric chloride for 10 minutes and then thoroughly washed in doubled distilled water. Then the fruit was cut open and then the embryo was extracted placed on under aseptic conditions in the laminar air flow.

The medium was autoclaved at 15 psi for 15-20 min. and allowed to cool before use. The embryos were transferred to 100 ml. Capacity plastic capped glass jars containing 20ml

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of medium incubated and placed under 16 hours photoperiod at 25 ± 1°C. Embryos with plumule initiated were acclimatized (while still inside the culture jar) by placing them at ambient room condition (28-32°C) for one week proir to potting. Then propagules were taken out of culture jar and roots were washed with tap water to remove adhering agar and the propagules were transferred to clay pots (6 cm. dia.) and standard Hoagland's (nutrient) solution was provided regularly to invigorate the seedlings for 30 days. Potted plants were covered with transparent plastic sheet to prevent desiccation and placed in net house. Then those invigorated seedlings were transferred to the original natural habitat of Mangrove *i.e.*, Sunderbans for afforestation programme. Plantation was done in plots with ridges and furrows. The spacing maintained was line to line 2 metre apart and plant to plant 2 metre distance. The seedlings were placed in furrow becuase the salinity normally more on ridges. Per cent of survival of seedlings were assessed after one month of plantation.

MS stock solution composition :

Chemicals	Mg/L	5 x (gm/L)	10 x (gm/L)
MS-I :			
NH_4NO_3	1650	8.25	16.5
KNO_3	1900	9.5	19
MS-II :			
$MgSO_4, 7H_2O$	370	1.85	3.7
$MnSO_4, 4H_2O$	22.3	0.1115	0.223
$ZnSO_4, 7H_2O$	8.6	0.043	0.086
$CuSO_4, 5H_2O$	0.025	0.000125	0.00025
MS-III :			
$CaCl_2, 2H_2O$	440	2.2	4.4
KI	0.83	0.00415	0.0083
$CoCl_2,6H_2O$	0.025	0.000125	0.00025
MS-IV :			
KH_2PO_4	170	0.85	1.7
H_3BO_3	6.2	0.031	0.062
$Na_2MoO_4, 2H_2O$	0.25	0.00125	0.0025
MS-V :			
NaFeEDTA chelate	...	0.1835	0.367
MS-VI :			
Glycine	2	0.01	0.02
Vitamin Stock :			
Nicotinic acid	0.5	0.0025	0.005
Pyridoxine HCl	0.5	0.0025	0.005
Thiamine HCl	0.1	0.005	0.001
Myo inositol	100	0.5	1
Agar	- 8 gm/l		
Sucrose	- 20 gm/l		
BAP	- 1 mg/l		

Note : The pH of the final MS was adjusted to 5.8.

RESULTS AND DISCUSSION

The fruits of *Ceriops decandra* were collected from Sunderban areas of West Bengal at different days of pollination and it was found that after 15 days of pollination the fruits were at right stage for embryo extraction. At that stage the average fruit size was 23 ± 01 mm. (Table 1) and extracted embryo (Fig 1A) and size was only 15 ± 0.3 mm. The seed moisture content was measured to be 65 ± 0.23 per cent indicating; Recalcitrant nature of seed. In *Ceriops* The germination was found to be viviparous in nature and there was continuous growth of embryo just after pollination. The average weight of the fruit was 850.00 ± 0.11 mg. Whereas the extracted embryo weighed only 120.00 ± 0.21 mg. This indicates that the embryo was only 14.12 per cent of the fruit. Small embryo, more seed moisture per cent were two important characters of recalcitrant type of seed. Whenever there was any desiccation and reduction in seed moisture content there was great reduction in *Ceriops* seed viability, high salinity and pollution were the other factors which reduced the per cent of seedling survibility of *Ceriops* and found to be only 61.00 ± 0.13 per cent (Table 2). The embryos at specific stage after embryo culture specially in MS-VI medium with BAP @ 1 mg/l gave best result for 3 weeks, the plumular leaves were initiate (Fig. 1B) and then those tubes were placed in ambient room temperature (32 ± 1°C), where the leaves started opening leaves (Fig. 1.C), and finally the seedlings were found with expanded leaves (Fig. 1.D). By embryo culture the per cent of embryo survived were increased upto 94 ± 1.02 per cent whereas at natural forest situation only 61 ± 0.13 per cent was recorded (Table 2). The seedlings grown by embryo culture were shifted to green house in earthen pots and were provided with standard Hoaglands solution, for invigoration twice a week and after 30 days of growth the seedlings were shifted to their natural habitat (Sunderban area) and afforestation programme was undertaken and plantation of those invigorated seedlings were done and the per cent of seedlings survived after embryo culture and invigoration treatment was recorded to be as high a 89 ± 0.74 per cent.

From this type of research work, the problem of seedling mortality at natural habitat can be reduced and per cent of seedling survival per unit area can be increased and directly helping for mangrove conservation and Management.

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Table 1 : Average fruit and embryo character of *Ceriops*.

Genus	Fruit size (mm)	Size of extracted embryo (mm)	Wt. of fruit (mg)	Wt. of extracted embryo (mg)	Per cent embryo seed	Seed moisture content (%)
Ceriops	23 ± 0.1	15 ± 0.3	850.00 ± 0.21	120.00 ± 0.21	14.12%	65.00 ± 0.23

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Table 2 : Average per cent of seedling survival by natural seed growth in forest by embryo culture and in natural environment after embryo culture.

Genus	Per cent of embryo survival by natural seedling growth in forest	Per cent of embryo survived by Embryo culture	Per cent of seedling survived at natural forest, after embryo culture
Ceriops	61 ± 0.13	94 ± 1.02	89 ± 0.74

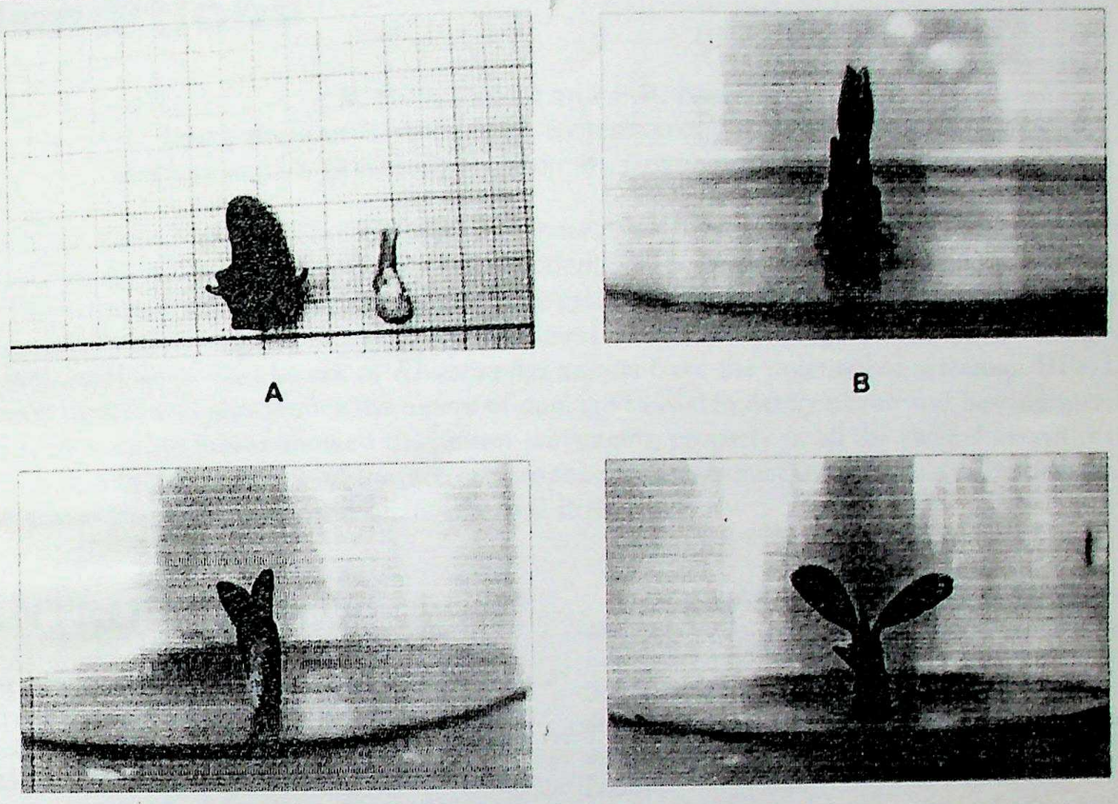

Fig 1 :
A = Fruit & extracted embryo of *Ceriops*.
B = Embryo of *Ceriops* with plumule initiation.
C = Embryo showing bract and first two leaves.
D = Embryo at final stage with two expanded leaves.

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Free Radical Scavenging Ability of *Rhinacanthus nasutus* Leaves Against Oxidative Damage Induced under *In Vitro* Conditions

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Antioxidants in traditional medicine claimed therapeutic properties for scavenging oxygen free radicals. Oxidative stress results from the depletion of antioxidants in the body either due to malnutrition or due to excessive production of reactive oxygen species. The main objective of the study was to analyze the radical scavenging ability of different solvent extracts of *Rhinacanthus nasutus* leaves against oxidative damage induced under *in vitro* conditions. The leaves of *Rhinacanthus nasutus* have the potential to scavenge DPPH, ABTS, and hydroxyl radical and also reduce the extent of damage caused to deoxy ribose and herring sperm DNA. Methanol extract of the leaves showed maximum scavenging property in all the radical assays performed. This may be due to the presence of the active compounds that extract into methanol, thereby exhibiting maximum scavenging effect against oxidant-induced stress.

INTRODUCTION

Focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems (Dahanukar *et al.*, 2000). Many herbal plants contain antioxidant compounds and these compounds protect cells against the damaging effects of ROS (Dasgupta and De, 2004). Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infectious and degenerative diseases (Park *et al.*, 2006).

Reactive oxygen species are highly oxidative substances, which may attack almost all cellular components and materials, especially the proteins, lipids and DNA of healthy cells. Reactive oxygen species such as superoxide radical (O_2^-), hydroxyl radical (OH), lipid radical (RH^-), lipid alkoxy radical (LOO^-) and lipid hydroperoxide (LOOH) show toxic effects when in contact with tissues of many organisms (Ekanayake *et al.*, 2005).

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant network in the body (Ghoghari *et al.*, 2006). Free radicals play an important role in tissue damage and pathological events. They are considered to be the cause of several health problems and chronic diseases such as cancer, cardiovascular diseases, arthritis and neurodegenerative disorders like Parkinson and Alzheimer's inflammation (Tepe *et al.*, 2005). Free radicals can affect food quality by reducing its nutritional content thereby promoting the development of food deterioration (Anagnostopoulou *et al.*, 2006).

Oxidative damage to cellular DNA can lead to mutations and may therefore play an important role in the initiation and progression of multistage carcinogenesis (Waris and Ahsan, 2006). Oxidative stress has been implicated in the pathology of many diseases including diabetes, cardiovascular disease, inflammatory conditions and cancer and aging (Malpure *et al.*, 2006).

MATERIAL AND METHODS

PREPARATION OF PLANT EXTRACTS

Aqueous, methanolic and chloroform extracts of the leaves of *Rhinacanthus nasutus* were prepared (1g/ml). The organic extracts (methanol or chloroform) were dried at 60°C protected from light. The residue was weighed and dissolved in dimethyl sulphoxide (DMSO) to obtain a final concentration of 20 mg/5µl. Aqueous extract was prepared fresh when experiments were performed. The different extracts were prepared in order to know the active components that are responsible for antioxidant activity.

The radical scavenging effects of the leaf extracts were analysed against DPPH (2, 2- diphenyl - 2 - picryl hydrazyl hydrate) (Mensor *et al.*, 2001) and ABTS (Azino bis thiazoline sulphonic acid) (Shirwaikar *et al.* 2006) radicals and antioxidant potential against the non-radical oxidant H_2O_2 (Ruch *et al.*, 1989).

The protective effects of *Rhinacanthus nasutus* leaf extracts on oxidative damage as inflicted upon deoxyribose which is a measure of hydroxyl radical scavenging activity

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(Elizabeth and Rao., 1990) and herring sperm DNA (commercially available - 0.5 mg/ml of Tris buffer) (Aeschlach *et al.*, 1994) were analysed. The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated with Fe^{3+}/ ascorbate/ EDTA/ H_2O_2 system. The hydroxyl radicals attack deoxyribose, which eventually result in TBARS formation.

The reaction mixture (0.5 ml) contained herring sperm DNA (0.5mg/ml of Tris buffer), H_2O_2 (0.05mg/ml), NaCl (5mM), $FeCl_3$ (50µM) and leaf extract (10µ l containing 10mg of extract prepared in Tris buffer (10mM, pH 7.4), L-ascorbic acid (0.5ml) was used as a positive control. The mixture was incubated at 37°C. The reaction was terminated by the addition of 0.05ml of 0.1M EDTA. The colour was developed by adding 0.5 ml of thiobarbituric acid followed by heating at 37°C for 15 minutes. After centrifugation, the extent of DNA damage was measured by the increase in absorbance at 532 nm.

RESULTS

Different radical scavenging assays were employed to assess the radical scavenging and protective effects of different leaf extracts of *Rhinacanthus nasutus* against oxidative DNA damage induced *in vitro*. The per cent scavenging activity of the different extracts is shown in Fig.1. DPPH scavenging ability has been used in many studies to evaluate the antioxidant potential of medicinal plants (Aquil *et al.*, 2006). Among the three extracts analysed, the methanolic extract of *R. nasutus* leaves showed its powerful DPPH scavenging effect followed by aqueous extract and a least scavenging effect was obtained from the chloroform extract as depicted in Fig.1.

ABTS radical cation is a rapid and reliable method which is used to measure total radical scavenging activity of many substances. All the extracts could scavenge the ABTS radical to different extent. Methanolic extract was the most effective followed by chloroform extract and then aqueous extract.

All the three extracts of *R. nasutus* exhibited strong hydrogen peroxide scavenging activity.

The protective effects of *Rhinacanthus nasutus* leaf extracts on oxidative damage inflicted upon Deoxy ribose and Herring sperm DNA were analyzed by means of extent of Thiobarbituric acid reactive substances (TBARS) produced and the results are presented and discussed below in Fig. 2 and Fig. 3 respectively.

The amount of TBARS formed in the group treated with H_2O_2 alone was fixed as 100% damage and the extent of the other groups were calculated relative to this value. The damage caused to deoxy ribose by H_2O_2 was very effectively counteracted by three different leaf extracts of *Rhinacanthus nasutus*.

The ultimate target of persistent oxidant moieties are the DNA molecules, damage to which can lead to severe consequences in the cell (Ani *et al.*, 2006).

H_2O_2 caused the maximum damage to Herring sperm DNA, The extent of damage decreased in a significant manner in the presence of different leaf extracts of *Rhinacanthus nasutus*. The maximum protection was offered by the methanolic extract followed by aqueous extract as reflected by the reduction in the per cent TBARS produced.

DISCUSSION

Zhang and Hamauzu (2003) have reported that DPPH radical scavenging activity in the methanol extracts was higher than that of the phenolic extracts. This is likely due to a high level of ascorbic acid remaining in the methanol extracts.

Methanolic extract of *Aspidium citutarium* rhizome containing tannins and phenolic compounds was found to possess excellent antiradical activity by quenching DPPH radical (Ghoghari *et al.*, 2006). Ethyl acetate and chloroform extracts of Broccoli seeds were considerable less effective as radical scavenger compared to methanolic extracts (Chuanphongpanish *et al.*, 2006).

Hydrogen peroxide is a strong oxidant that can result in extensive damage ultimately causing cellular death. Thus, agents that can scavenge or degrade hydrogen peroxide are believed to possess strong antioxidant activity. Gulcin *et al.*, (2007) have reported that methanol extract and ethyl acetate extract have an effective hydrogen peroxide scavenging activity.

Hydroxyl radicals have been implicated as highly damaging species in free radical pathology. This radical has the capacity to join nucleotides in DNA, cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity (Murthy *et al.*, 2006).

Methanolic extracts were able to suppress the generation of hydroxyl radicals as reflected by the reduction in degradation of deoxy ribose and it showed excellent inhibition indicating that it could strongly scavenge hydroxyl radicals. The plant phenolic compounds such as genistein and resveratrol showed its ability by inhibiting the damage induced by Reactive oxygen species in phi x-174 plasmid DNA. They were also effective against hydrogen peroxide induced oxidative DNA (Win *et al.*, 2002).

Thus, the plant leaves serve as natural sources to develop the free radical attack in biological and food systems and also possess therapeutic effects.

The present study revealed that *Rhinacanthus nasutus* could inhibit hydrogen peroxide induced damage to cellular DNA, supporting a protective effect of *Rhinacanthus nasutus* against oxidative damage.

CONCLUSION

The overall results observed for all the parameters showed that the leaves of *Rhinacanthus nasutus* possess

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Fig. 1 : FREEE RADICAL SCAVENGING EFFECTS OF *Rhinacanthus nasutus* leaves

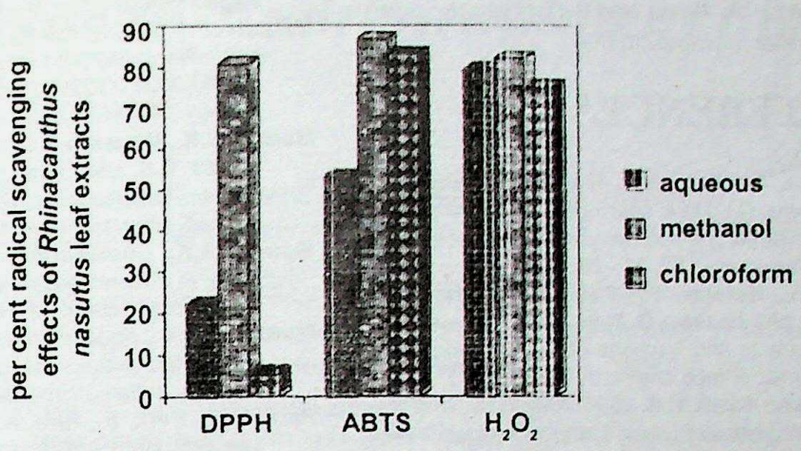


Fig.2 : EFFECT OF *RHINACANTHUS* LEAVES ON OXIDANT INDUCED DAMAGE TO DEOXY RIBOSE

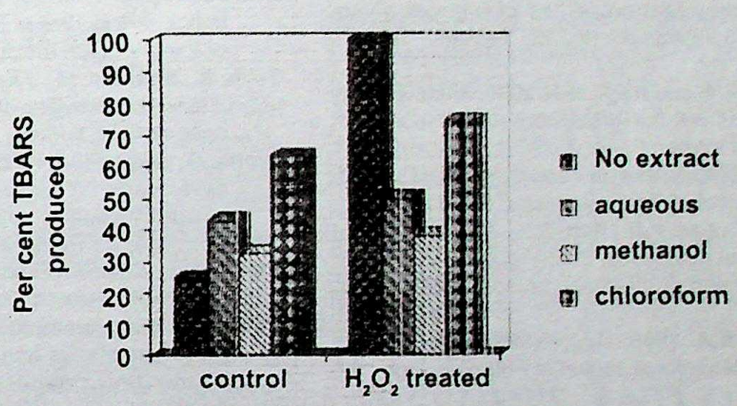
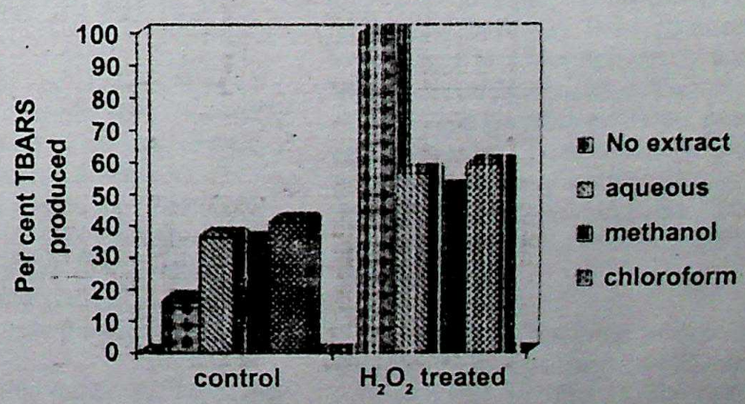


Fig.3. : EFFECT OF *RHINACANTHUS* LEAVES ON OXIDANT INDUCED DAMAGE TO HERRING SPERM DNA



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strong radical scavenging, antioxidant potential and DNA-protective effects thus validates the use of *Rhinacanthus nasutus* leaves in medicinal preparations for diseases and disorders caused by oxidative stress and it can be concluded that the plant has potential therapeutic use.

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Standardization of Micropropagation Techniques in *Andrographis echioides* (L.) Nees.

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Micropropagation of *Andrographis echioides* (L.) Nees was carried out to evaluate *in vitro* response of different explants / media for regeneration and also to standardize the direct regeneration procedure. Among the various explants, shoot tips responded positively for shoot induction. MS medium fortified with the plant growth hormone, 6-benzyl adenine (= benzyl aminopurine = BAP)(2.5 mgl^{-1}) was found highly responsive for shoot induction. The multiple shoot induction was achieved in MS medium + BAP (3.0 mgl^{-1}) and for shoot elongation medium with BAP (2.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1}) was found better. Rooting was best (94.85%) in ½ MS + IAA 0.5 mgl^{-1} + IBA 1.0 mgl^{-1}. Pot mixture containing vermiculite + red earth + sand (1:1:1) was found optimum for hardening.

INTRODUCTION

Andrographis echioides (Gopuramthanki) is one of the important medicinal plant species belonging to the family Acanthaceae. *Justicia echioides* L. and *Indoneesiella echioides* (L.) Sreemadh are the synonyms *A. echioides*, while other related species *A. alata*, *A. echioides*, *A . paniculata*, *A. serpyllifolia* and *A. wightiana* are gaining importance. Following the Indian systems of medicine, *A. echioides* is being used against blood cancer. The leaf extract is recommended for oral consumption. Traditionally, the plant has also been used as febrifuge, bitter tonic, astringent, anodyne and for dysentery, cholera and diabetes. The ethanol extract of this plant is recently used as diuretic and in sluggishness of liver and jaundice. Earlier research work on tissue culture of this plant is very meager. Hence in the present work its *in vitro* culture has been studied.

MATERIAL AND METHODS

For micropropagation, the explants of shoot tips (1.5 - 2 cm), nodal segments (2 - 2.5 cm), leaf bits (0.5 - 1.0 cm^2), root bits (1.0 - 2.0 cm) and stem bits (1 - 1.5 cm) were collected from healthy mother plants of *A. echioides* and trimmed off to required sizes with a sterilized knife before inoculation. The explants were rinsed with liquid detergent for five minutes and then rinsed with distilled water for four times. Prior to inoculaion, explants were sterilized with ethyl alcohol (70%) for 25 seconds and were rinsed with 0.1 per cent mercuric chloride.

For shoot formation, the explants were cultured on MS basal medium alone and also on the medium in combination with BAP (0.5 to 4.0 mgl^{-1}). For multiple shoot production, the explants were inoculated on MS basal medium supplemented with BAP (1.0 - 5.0 mgl^{-1}). The shoot elongation was tried on MS basal medium alone and also on medium with BAP (1.0 - 4.0 mgl^{-1}) and GA_3 (0.5 - 1.0 mgl^{-1}) combinations. The individual microshoots obtained from the shoot induction media were transferred to ½ MS basal medium (Control), IAA (0.5 to 1.0 mgl^{-1}), IBA (0.5 to 1.0 mgl^{-1}) and their combinations for root induction. Activated charcoal (200 mgl^{-1}) was added to all the treatment combinations. The plantlets with well-developed shoots and roots were transferred to different treatments of hardening media with vermiculite, red soil, sand individually and in combination.

RESULTS AND DISCUSSION

Explant standardization and shoot induction : The explants of shoot tips, nodal segments, stem bits, leaf bits and root bits were sourced from the stock plant of *A. echioides* maintained for the purpose of mircopropagation. Among them, shoot tips gave significantly highest response (85.72 %) followed by nodal segment (70. 63 %). The high endogenous auxin content in both these explants apparently promoted cell division and thereby good regeneration. Explants that have a rudimentary or organized structure (shoot tip, nodal segment) are the most responsive and pose the least problems in respect of an organized structure which it will suffice to reveal in an appropriate medium (Haripriya, 2003). This response might be also due to the higher meristematic activity (Suryanarmada, 2000).

The best response to direct shoot regeneration from shoot tips was observed on MS medium supplemented with BAP (2.5 mgl^{-1}) (Table 1). The response to shoot induction decreased as the concentration of BAP increased. The decrease in shoot production at higher concentration of BAP may be due to the inhibition of shoot initiation or induction of

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calli. Direct organogenesis helps more to retain clonal fidelity (Broertjes and Keen, 1980) than to shoot production through callus.

Multiple shoot induction: The multiple shoots were observed more on medium containing BAP (3.0 mgl^{-1}) (Table 1). Number of multiple shoots induced was higher and quicker at higher concentration of BAP which did not, however, influence shoot length. But reduction in shoot length due to higher concentration of BAP was reported in the case of *Rauwolfia tetraphylla* by Ghosh and Banerjee (2003).

Shoot elongation : The effect of GA_3 in combination with BAP was studied for shoot elongation. The longest shoots were produced on the medium containing BAP (2.0 mgl^{-1}) and GA_3 (1.0 mgl^{-1}) treatment in 8. 97 days. GA_3 is known to stimulate cell elongation and cell-wall plasticity. Cell division was stimulated in the shoot apex especially in the more basal meristematic cells, from which develops the long files of cortex and pith cells. GA_3 treatment helps in both the transport of potassium ions and increase in the number of mitotic figures throughout the meristematic zone (Sachs, 1965). They also promote cell growth because they increase hydrolysis of starch ans sucrose into glucose and fructose molecules (Salisbury and Ross, 1986). Similar trend of shoot elongation was found in *Centella asiatica* (Panimalar, 2005) and *Andrographis paniculata* (Haripriya, 2003).

Rooting : The medium containing ½ MS + IAA 0.5 mgl^{-1} + IBA 1.0 mgl^{-1} gave highest (94.85%) and earliest (11.45 days) rooting (Table 5) producing longer (3.73 cm) and more number of roots (10.75) (Table 2). A mixture of more than one auxin could be effective for root induction as auxin is implicated in vascular differentiation (George and Sherrington, 1984) and (Roy *et al.*, 1995).

Hardening : The natural habitat of *Andrographis echioides* is commonly in the dry tracts. Hence a potting mixture with high water holding capacity was found unsuitable for survival of the *in vitro* derived plantlet. Low survival percentage in hardening media containing vermiculite alone might be bue to high water-holding capacity of the media (Saraswathi, 2006), Vermiculite, red earth and sand in pot mixture had significant effect on survival of plants during hardening (Table 3). Combination of vermiculite + red earth + sand performed better in hardening of *in vitro* derived plantlets with least mortality and highest survival percentages (29.72% and 60.38 % respectively).

Table 1 : Effect of BAP on *in vitro* shoot induction and proliferation from shoot tip explants of *Andrographis echioides*.

Treatments	BAP (mgl^{-1})	Shoot induction (%)	Multiple shoot induction (%)
T_1	MS basal	0.00 (0.64)	0.00 (0.64)
T_2	0.5	20.21 (26.70)	-
T_3	1.0	37.45 (37.73)	0.00 (0.64)
T_4	1.5	61.65 (51.76)	-
T_5	2.0	63.67 (52.96)	65.12 (53.82)
T_6	**2.5**	**83.22 (66.10)**	-
T_7	3.0	74.56 (59.81)	**78.62 (62.52)**
T_8	3.5	67.18 (55.10)	-
T_9	4.0	43.33 (41.16)	67.64 (55.35)
T_{10}	4.5	-	-
T_{11}	5.0	-	60.33 (50.97)
	Mean	50.14 (43.55)	45.29 (37.32)
	SEd	2.431	1.448
	CD (0.05)	5.107	3.115
	CD (0.01)	6.998	4.423

Values in parentheses are arcsine-transformed.

SUMMARY AND CONCLUSION

Among the five explants of *A. echioides*, *viz*, shoot tip, nodal segment, stem bit, leaf bit and root bit tried for shoot induction, Shoot tip explants responded positively. Highest (83.22 %) and earliest (12.55 days) shoot induction was recorded on MS medium fortified with BAP (2.5 mgl^{-1}). Multiple shoots were observed more in the medium containing BAP (3.0 mgl^{-1}). For shoot elongation, BAP (2.0 mgl^{-1}) + GA_3 (1.0 mgl^{-1}) combination was found to be better. The medium containing ½ MS + IAA 0.5 mgl^{-1} + IBA 1.0 mgl^{-1} gave highest (94.85 %) and earliest (11.45 days) rooting as well as longer (3.73 cm) and more number of roots (10.75). The treatment combination of vermiculite + red earth + sand performed better in hardening of *in vitro* derived plantlets with least mortality and highest survival percentages (29.72 % and 60.38 % respectively).

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Table 2 : Effect of growth regulators on *in virto* rooting percentage and days taken for rooting in *Andrographis echioides*.

Treatments	Growth regulators (mgl^{-1})		Rooting (%)	Days taken for rooting	Number of roots / plant	Root length (cm)
	IAA	IBA				
T_1	½ MS Basal	-	0.00 (0.64)	0.00 (0.64)	0.00 (0.64)	0.00 (0.64)
T_2	0.5	-	20.50 (26.92)	3.85 (11.31)	3.85 (11.31)	1.50 (7.03)
T_3	1.0	-	46.43 (42.95)	4.45 (12.17)	4.45 (12.17)	1.98 (8.09)
T_4	-	0.5	70.37 (57.04)	6.45 (14.71)	6.45 (14.71)	2.10 (8.33)
T_5	-	1.0	88.55 (70.37)	6.90 (15.22)	6.90 (15.22)	2.70 (9.45)
T_6	0.5	0.5	90.50 (72.26)	8.02 (16.44)	8.02 (16.44)	3.07 (10.08)
T_7	**0.5**	**1.0**	**94.85 (77.60)**	**10.75 (19.13)**	**10.75 (19.13)**	**3.73 (11.13)**
T_8	1.0	0.5	88.52 (70.34)	7.05 (15.39)	7.05 (15.39)	2.70 (10.70)
T_9	1.0	1.0	90.83 (72.60)	13.05	8.43 (16.87)	3.45 (10.70)
Mean			65.62 (54.52)	16.05	6.21 (13.54)	2.36 (8.32)
SEd			2.448	0.551	0.425	0.292
CD (0.05)			5.144	1.157	0.893	0.613
CD (0.01)			7.049	1.586	1.223	0.840

Values in parentheses are arcsine-transformed.

Table 3 : Effect of pot mixtures on hardening of *in vitro* derived plantlets of *Andrographis echioides*.

Treatments	Pot mixture	Survival (%)	Mortality (%)
T_1	Vermiculite	41.67	48.37
T_2	Red earth	50.50	42.00
T_3	Sand	52.01	38.06
T_4	**Vermiculite + red earth + sand**	**60.38**	**29.72**
Mean		51.14	39.54
SEd		0.298	0.463
CD (0.05)		0.648	1.009
CD (0.01)		0.909	1.414

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In Vitro Somatic Embryogenesis of *Mucuna pruriens* (L.)

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In *Mucuna pruriens* plant, leaf and petiole were used as explants for callus induction in MS medium with different concentrations of 2,4-D, NAA for callus induction. After the callus were transferred to MS medium containing various concentrations of 2,4-D, Picloram, BA, GA_3 for somatic embryo induction and glutamine for somatic embryo maturation.

Keywords : Callus culture, *in vitro*, somatic embryogenesis, *Mucuna pruriens*.

INTRODUCTION

Traditional herbal medicines have played a key role in the health system of many countries. Several thousand medicinal plants are being destroyed due to human neglect and industrialization, (Anonymous, 1993: Zeml, 1978). *Mucana pruriens* (L.) DC (Fabaceace) is an important medicinal plant possessing valuable medicinal properties, (Caius, 1989; Pandey, 1998 and 1999). It is commonly known as cow - itch plant, Nescafe (or velvet bean, poonaikkali (cat - legged). *Mucuna pruriens* is an herbaceous twinging annual. The leaves are trifoliate leaflets are broadly ovate, elliptic and unequal at base. The flowers are purple in axillary, in September / November; fruits in January / April. The seeds are in black colour and 4-6 in a pod and ovoid shape (Sastry and Kavathekar, 1990; Agharkar, 1991; Verma *et al.*, 1993). Lectins which are receipts specific proteins also have been reported from the seed of *Mucuna pruriens* (Kaushik, 2009). Chemical constituents and medicinal utility of *Mucuna pruriens* has been reported in detail by Kaushik and Dhiman (2000).

MATERIAL AND METHODS

Mucuna pruriens seeds were collected from Periyar University Campus Salem and were grown in the herbal garden, Department of Plant Science, Bharathidasan University, Tiruchirappalli. The explants were excised (leaf, Petiole) from the field grown healthy plant (60-days old) for culturing. The explants were transferred to MS cultures medium supplemented with different growth regulators (2,4-D, NAA, Picloram) for callus induction, Cultures were maintined in a culture room at 25 ± 2°C under 16th Photo period provided by cool white fluorescent light ($35Em^2s^{-1}$) with 55-65 % relative humidity. Explants were placed horizantally on the medium.

Whitish yellow friable calli were transferred to induction medium containing various concentrations of 2,4-D, Picloram,

Table 1 : The composition of MS medium.

Components	Concentration (mg/l)
Macronutrients :	
KNO_3	1900
NH_4NO_3	1650
$CaCl_2.2H_2O$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	170
Micronutrients :	
$MnSO_4.4H_2O$	22.3
$ZnSO_4.7H_2O$	8.6
$CuSO_4.6H_2O$	0.025
KI	0.83
$Na_2MOO_4.2H_2O$	0.25
$CoCl_2.6H_2O$	0.025
Vitamins :	
Myoinositol	100
Thiamine HCl	0.1
Nicotinic acid	0.5
Pyridoxine HCl	0.5
Glycine	4
Boric acid :	
H_3BO_3	6.2
Iron sources :	
$FeSO_4.7H_2O$	27.85
$Na_2EDTA\ 2H_2O$	37.25
Carbon sources	
Sucrose	30g/l versity
Solidifying agent	
Agar	8g/l
pH	5.6 – 5.8
Growth regulators :	
Auxins	
1-Naphthalene acetic acid (NAA)	(0.1 - 5.0 mg/l)
2,4 - Dichlrophenoxy acetic acid	(0.1 - 5.0 mg/l)
Picloram (Pic)	(0.1 - 5.0 mg/l)
Cytokinin	
6- Benzylaminopurine (BA)	(0.1 - 5.0 mg/l)
Kinetin (KN)	(0.1 - 3.0 mg/l)
Gibbrellic acid (GA_3)	(0.5 - 3.0 mg/l)

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Table 2 : Callus induction (%) from petiole explants of *Mucuna pruriens* grown on MS medium supplemented with 2,4-D, NAA, picloram after 15 days.

Growth regulators (mg/l)	Callus initiation (%)	Nature of Callus
2,4 - D		
1.0	71.0^{ef}	White Friable Callus
2.0	82.4^{a}	"
3.0	76.2^{cd}	"
4.0	70.4^{f}	"
5.0	64.2^{hi}	"
NAA		
1.0	60.0^{i}	White Friable Callus
2.0	68.0^{g}	"
3.0	72.4^{e}	"
4.0	65.2^{h}	"
5.0	62.6^{i}	"
Picloram		
1.0	76.2^{cd}	White Friable Callus
2.0	74.4^{e}	"
3.0	80.2^{b}	"
4.0	76.4^{c}	"
5.0	62.2^{k}	"

kinetin, BA and GA_3. At the end of 30 days, percentage of callus with somatic embryogenesis (calli with globular stage and later stages of embryos visible to naked eye and microscopically) was recorded.

RESULTS AND DISCUSSION

Callus Induction : Petiole and leaf explants were grown on MS medium containing 2,4-D, NAA and picloram for callus induction. In the two explants, Petiole showed the highiest percentage of callus induction than leaf. Among three auxins (2,4-D, NAA, Picloram), 2,4,-D induced the highest amount of white friable callus than others. In the early phase of the process, a mixture of white and friable callus was noted, the friable callus tending to become yellow in its later stage. Thereafter, it was necessary to subculture the callus for several times at 2-4 weeks intervals.

Somatic embryo induction : Somatic embryogenesis was induced from the callus grown on MS medium augmented with different concentrations of 2, 4-D and picloram either alone (or) in combination and glutamine. The highest percentage of somatic embrogenesis was observed on MS medium

Table 3 : Somatic embryo induction from petiole explants of *M.pruriens* grown on MS medium Supplemented with 2,4-D picloram and glutamine after 15 days of culture.

Growth regulators (mg/l)			% of response	Mean no.of Somatic embryos	Nature of Callus
2,4-D					
0.5			48.2^{m}	2.0^{j}	WFC
1.0			53.4^{k}	2.6^{hi}	"
3.0			65.2^{i}	3.0^{h}	"
5.0			51.2^{l}	2.8^{hi}	"
Picloram					
0.5			53.2^{kl}	2.0^{j}	YFC
1.0			62.2^{j}	3.2^{g}	"
3.0			75.0^{g}	4.2^{f}	"
5.0			68.2^{h}	4.0^{fg}	"
2,4-D	Picloriam				
3.0	0.5		72.6^{gh}	5.2^{ef}	WFC
3.0	1.0		81.2^{e}	6.3^{c}	"
3.0	3.0		70.4^{h}	5.4^{e}	"
3.0	5.0		64.2^{ij}	4.8^{f}	"
2,4-D	Picloram	Glutamine			
3.0	1.0	25	80.4^{ef}	5.4^{e}	WFC
3.0	1.0	50	84.2^{cd}	5.8^{e}	"
3.0	1.0	75	90.2^{b}	6.2^{cd}	"
3.0	1.0	100	94.6^{a}	7.8^{a}	"
3.0	1.0	125	86.7^{c}	7.2^{b}	"

Supplemented with 2,4-D (3.0mg/l), picloram (1.0mg/l) and L-glutamine (100mg/l)

Maturation : The somatic embryos (globular shaped) were transferred to maturation medium MS salts containing different concentration and combination of BA and GA_3. The addition of L-glutamine (100mg/l) enhanced the somatic embryo, maturation (heart shaped and bipolar).

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Table 4 : Somatic embryo maturation of *M.pruriens* grown On MS Containing BA and GA_3 after 20 days of Culture.

Growth regulators (mg/l)	Percentage of response	Mean no. of Somatic embryos
BA		
0.5	48.2^{g}	-
1.0	65.6^{d}	1.2^{c}
1.5	85.4^{a}	2.5^{a}
2.0	82.0^{ab}	-
2.5	78.2^{bc}	-
GA_3		
0.5	52.4	-
1.0	64.2	-
1.5	72.0	1.4^{b}
2.0	68.0	-
2.5	60.4	-

Note: Each value was the average of 10 replicates and repeated three times. Mean Seperation -Using Duncans Multiple Range Test (DMRT). means within column with different letters are Significant at the 5% level.

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Effect of Growth Regulators on Callus Initiation and Plantlet Regeneration from Leaf Explants in *Gerbera jamesonii* Bolus

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The maximum callus induction and growth in leaf explant was observed on Murashige and Skoog (MS) medium supplemented with 1.00 mg/l 2,4-D for callus initiation and fresh weight of callus. These callii were successfully regenerated on 1.0 and 1.5 mg/l BAP while direct rooting of callus was observed in 1.0 mg/l NAA. The significant results were found with 2.0 mg/l Kinetin and 0.5 mg/l BAP for initiation of shoots and 0.5 mg/l BAP for number of plantlets per explant. Maximum height of plantlets was found with 0.5 mg/l Kinetin while 1.5, 2.0 and 0.5 mg/l BAP + 0.5 mg/l Kinetin were found to induce more number of leaves per explant. These plants were successfully rooted with different auxins but IAA was found to promote root elongation. These rooted plantlets were successfully hardened and acclimatized after 21 days with 70-80 per cent success with carrier vermiculite.

Keywords : *Gerbera*, growth regulators, regeneration, *in vitro*

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INTRODUCTION

Gerbera is a valuable ornamental species grown as a potted plant as well as for its cut flowers. The most efficient *in vivo* method for vegetative propogation is by making rhizome cuttings. However, multiplication by this method is too slow to be commercially viable (Orlikowska *et al.* 1999). *In vitro* propagation is commonly used for rapid and large-scale multiplication (Palai *et al.*, 1998, Aswath and Chaudhary 2002, Zhang, 2002). Adventitious shoots in *Gerbera* have been regenerated from the callus using different explants (Barbosa *et al.* 1994, Parthasarathy *et al.* 1997, Le *et al,* 1999, Pasoda *et al.* 1999, Kumar *et al.* 2004). The regeneration from floral buds or capitulum has also been reported in *Gerbera* by many workers (Pierik *et al*, 1975, Laliberte *et al.* 1985, Barbosa *et al* 1994, Chen and Chen 2002). However, the percentage of explants with sprout formation with this method is low (Pierik *et al.* 1982). The regeneration of shoots from leaf blades was observed by Hedtrich (1985) during *in vitro* propagation of *Gerbera jamesonii* cv. Vulkan. In the present investigation, an attempt has been made to improve regeneration in *Gerbera* with different levels of auxins and cytokinins and to promote growth and development by using leaf sections as explant.

MATERIAL AND METHODS

Tender emerging leaves with petioles of *Gerbera* (*Gerbera jamesonii* Bolus) to be used as explants were collected from All India Coordinated Floriculture Improvement Project, NARP, Ganeshkhind, Pune. The gerbera used in this experiment is a cut flower cultivar with double type of flowers having red rays, transtlorets and a black disc. These explants were washed in running tap water and then washed thoroughly by adding a few drops of Tween-20. Then they were surface sterilized in a 0.1% mercuric chloride for five minutes followed by rinsing them three to four times with dobule distiled water inside the laminar flow. The cut pieces were used as explants and cultured on callus induction medium (CIM) comprising of Murashige and Skoog (MS) (1962) basal medium supplemented with different concentration of 2, 4-dichlorophenoxy acetic acid (2,4-D) along with 30gm/l sucrose and 0.8% agar. The pH of the medium were adjusted to 5.8 with 0.1 NaOH before autoclaving at 15 psi for 15 minutes. The data were recorded for days to callus initiation and fresh weight of callus (gm) after 65 days of incubation. Each treatment comprised of 40 explants with 8 pieces in one bottle. A total of three replications comprising of 5 bottles each and the data are the mean of the three replications. The cultures were maintained at 22 ± °C under cool florescent light intensity with 16 hrs light and 8 hrs dark photoperiod cycle. These 65 days old calli were transferred to shoot regeneration medium with MS + 1.0 mg/l BAP + 0.5 mg/l Kinetin. The regenerated shoots were subcultured every 25 days for further shoot formation. These shoots were than cultured on MS medium with different concentrations of cytokinin (Table 2). After the shoot formation these *in vitro* shoots were than kept for rooting in MS medium supplemented with different auxin levels. (Table 3). The data were recorded on different parameters and statistically analyzed by using the Completely Randomized Design (Panse and Sukatme 1985). The rooted plants were hardened and planted in 10 cm diameter pots containing different carriers and the survival percentage was recorded.

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RESULTS AND DISCUSSION

(A) 1. Effect of 2,4-D levels on callus initiation : Six different media compositions of 2,4-D levels were studied for callus initiation and fresh weight of callus in gerbera. The 2,4-D levels had significant effect on initiation of callus (Table 1). The number of days to initiation of callus varied from 6.33 to 10. 43 days. Minimum 6.33 days were required with 1.00 mg/l 2, 4-D, while maximum 10.43 days were required with 2.0 mg/l 2,4-D. Fresh weight ranged from 2.62 to 4.99 gm. The maximum fresh weight of callus was observed with 1.00 mg/l 2,4-D while minimum was observed with 2.00 mg/l 2,4-D followed by 0.25 mg/l 2,4-D. This was due to the inhibitory effect of 2,4-D. In all the levels of 2,4-D tested the friable type of callus was initiated. Bonga (1987) reported that the type of explant used for induction of callus mainly depends upon the juvenility of the explants. Kumar and Kanwar (2004) found maximum callus induction with 1.5 and 2 mg/l 2,4-D in gerbera leaf petiole explants. Similar finding were also reported by Kumar *et al* (2006).

2. Regeneration of plantlets from callii : The different combinations of cytokinins and auxin levels in MS medium were tried to regenerate the plantlets from the callii. Most of the callii did not survive and turned brown when put in the medium containing cytokinins and auxins. In MS medium containing 1.0 and 1.5 mg/l BAP maximum plantlet regeneration was observed. In medium supplemented with 0.5, 1.0 and 2.0 mg/l NAA direct rootings were observed in the cultures. These roots were thick and the cultures did not survive when transferred to the shoot initiation medium containing 0.5, 1.0 and 1.5 mg/l BAP for shoot induction. Similar findings were also reported by Kumar *et al.*, (2004) who obtained regererated shoots from callus 1.5, 2.0 and 3.0 mg/l BAP but failed to produce shoots in NAA and IAA containing medium.

(B) Effect of cytokinins on shoot initiation and multiplication in gerbera : The growth regulator had highly significant effect on time taken for initiation of shoots, number on plantlets per explants, plant height and number of leaves per plant (Table 2). The mean variation of days to shoot initiation ranged from 4.67 to 6.67 days. Minimum 4.67 days were required with 2.0 mg/l kinetin and 0.5 mg/l BAP, while the maximum days (6.67 days) were required by 1 mg/l kinetin and 2.0 mg/l BAP + kinetin for initiation of shoots. Initiation of shoot was delayed when kinetin was added to BAP.

Number of plantlets per explant ranged from 4.33 to 16.00. The maximum number of plantlets per explants was observed with 0.5 mg/l BAP while the minimum number was observed in 0.5 mg/l kinetin. With increased concentration of 0.5 BAP and 0.5 mg/l BAP + kinetin there was decrease in multiple plantlet formation, while it was reverse in case of kinetin levels. The multiple shoot formation was prominent when BAP concentration in medium was low. Pasoda *et al.*, (1999) found best regenerated shoots at 1.0 and 2.0 ppm BAP. Higher concentration reduced the shoot proliferation in gerbera. The range for plant height varied from 1.83 to 5.17 cm maximum with 0.5 mg/l kinetin and minimum with 2.0 mg/l BAP followed by 1.5 mg/l BAP. The plant height decreased as the concentration of medium was increased in case of kinetin and BAP levels. Similar trand was also observed in BAP + kinetin combination levels also. The mean variation for number of leaves per explant ranged from 3.0 to 4.33 leaves. The maximum number of leaves per plant were observed with growth regulator levels of 1.5 mg/l BAP, 2.0 mg/l BAP and 0.5 mg/l BAP + kinetin. The minimum number of leaves was observed with growth regulator level of 0.5 mg/l BAP and kinetin, 1.5 and 2.0 mg/l BAP + kinetin. The effectiveness of BAP and kinetin depends on the concentration of the active sites for biochemical action. This in turn depends on the interrelated process, including efficiency of uptake, translocation within the plants and accumulation at the active sites. The BAP cytokinin showed more number of plantlets per explant and leaves but reduced plant height while in kinetinless number of plantlets per explant apparently contributed to the plant height. Nafees *et at.*, (2009) found that the multiplication rates of gerbera was occasionally high up to 30 shoots depending upon the cytokinin content of the medium.

(C) *In vitro* rooting in gerbera : It was found that the NAA, IAA and IBA levels had significant effects on days to root initiation, number of roots and root length (Table-3). The mean response ranged from 9.33 to 13.33 for days to root initiation. The minimum and maximum days were required with 0.5 mg/l IAA and 2mg/l IBA, respectively for root initiation. In IAA levels it was observed that it required less days for root initiation as compared to NAA and IBA levels. The mean variation for number of roots per plant ranged from 3.33 to 6.33 roots. The maximum number of roots per plant was observed in 1.5 mg/l NAA and 1 mg/l NAA along with 2 mg/l IBA, respectively. The lowest number of roots was found in 2.0 mg/l IAA level as compared to NAA and IBA concentrations.

The range for root length varied from 3.67 to 6.83 cm. The maximum root length was observed in 2mg/l IAA, while the minimum root length was found in 2mg/l IBA and 0.5 mg/l IAA. The IAA concentration levels were much superior for root length as compared to NAA and IBA levels. IAA is a naturally occurring auxin while NAA and IBA are synthetically derived. The synthetically derived auxins appeared to be more effective and hence contributed to more number of roots and reduced the root length than IAA. Though the roots were less in IAA it contributed to the root length. In NAA a little of callus formation was observed at the base of root formation which was thick and hard. This character was undesirable as during hardening of plants this led to root rot in such type of plants.

(D) Hardening and acclimatization : The *in vitro* regenerated plantlets with good shoot and root system were taken out from the rooting medium and washed thoroughly with tap water. These plantlets were potted in community pots for primary hardening containing different substrates *viz.* (I) soilrite (2) vermiculite (3) coco-peat (4) soil (5) soilrite + coco-peat (1:1 v/v) (6) soil + coco-peat (1:1 v/v). The plantlets were kept in polyethylene tunnel for 21 days for primary hardening with intermittent misting to maintain high humidity around them. From the results it was seen that among the different carriers tested for successful transfer of *in vitro* raised plantlets, higher survival rate of (90%) was recorded in the carrier vermiculite followed by soilrite (65%), soilrite + coco-peat (30 %) while

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Table 1 : Effect of different 2, 4-D levels on days to callus initiation and fresh weight of callus in *Gerbera jamesonii* Bolus.

Sr. No.	MS+2, 4-D level (mg/l)	Days to callus initiation	Fresh weight of callus (gm) After 65 days of incubation	Type of callus
1.	MS + 0.25	8.55	2.68	Friable
2.	MS + 0.50	8.01	4.25	Friable
3.	MS + 0.75	7.87	4.90	Friable
4.	MS + 1.00	6.33	4.99	Friable
5.	MS + 1.50	8.76	3.29	Friable
6.	MS + 2.00	10.43	2.62	Friable
	Range SE + CD (5%)	6.33 to 10.43 0.43 1.33	2.62 to 4.99 0.11 0.34	

Table 2 : Effect of different levels of BAP, kinetin, BAP + kinetin on days to initiation of shoots, number of plantlets per explant, plant height and number of leaves per plant in gerbera.

Sr. No.	MS + growth regulator	Days to initiation of shoots	Number of plantlets per explant	Plant height (cm)	Average Number of leaves per plant
1.	MS + 0.5 mg/l kinetin	5.67	4.33	5.17	3.00
2.	MS + 1.0 mg/l kinetin	6.67	4.67	4.67	3.33
3.	MS + 1.5 mg/l kinetin	5.33	5.67	4.00	3.33
4.	MS + 2.0 mg/l kinetin	4.67	7.67	3.00	3.67
5.	MS + 0.5 mg/l BAP	4.67	16.00	3.33	3.00
6.	MS + 1.0 mg/l BAP	6.00	14.67	2.83	3.67
7.	MS + 1.5 mg/l BAP	6.00	10.67	2.33	4.33
8.	MS + 2.0 mg/l BAP	6.00	8.33	1.83	4.33
9.	MS + 0.5 mg/l BAP + kinetin	5.00	11.33	3.17	4.33
10.	MS + 1.0 mg/l BAP + kinetin	6.00	10.67	3.67	3.33
11.	MS + 1.5 mg/l BAP + kinetin	6.33	9.33	3.33	3.00
12.	MS + 2.0 mg/l BAP + kinetin	6.67	9.00	2.33	3.00
	Range SE + CD (5%)	4.67 to 6.67 0.45 1.31	4.33 to 16.0 0.48 1.40	1.83 to 5.17 0.25 0.74	3.00 to 4.33 0.36 1.05

coco-peat, soil and soil + coco-peat showed 100 per cent mortality of plants due to non-drainage of water and decay. Pasoda *et al.*, (1999) reported higher mortility of plants raised in sand medium. Similarly, Ajitabh *et al.* (2006) found vermiculite as the best carrier for *in vitro* raised plantlets of *Dianthus caryophyllus* as compared to vermiculite + sand and vermiculite + coco-peat.

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Table 3 : The effect of different auxin levels on days to root initiation, number of roots per plant and root length in gerbera.

Sr. No.	MS + Growth regulator	Days to root initiation	Average Number of roots per plant	Root length (cm)
1.	MS + 0.5 mg/l NAA	11.67	5.00	5.50
2.	MS + 1.0 mg/l NAA	12.33	6.00	4.83
3.	MS + 1.5 mg/l NAA	12.33	6.33	4.33
4.	MS + 2.0 mg/l NAA	12.67	5.67	3.67
5.	MS + 0.5 mg/l IAA	9.33	4.00	6.17
6.	MS + 1.0 mg/l IAA	10.00	3.67	6.67
7.	MS + 1.5 mg/l IAA	10.33	3.67	6.67
8.	MS + 2.0 mg/l IAA	10.67	3.33	6.83
9.	MS + 0.5 mg/l IBA	11.00	4.67	5.67
10.	MS + 1.0 mg/l IBA	12.67	5.00	4.50
11.	MS + 1.5 mg/l IBA	12.67	5.67	4.17
12.	MS + 2.0 mg/l IBA	13.33	6.00	3.67
	Range	9.33 to 13.33	3.33 to 6.33	3.67 to 6.83
	SE +	0.38	0.48	0.29
	CD (5%)	1.12	1.40	0.85

Plate 1

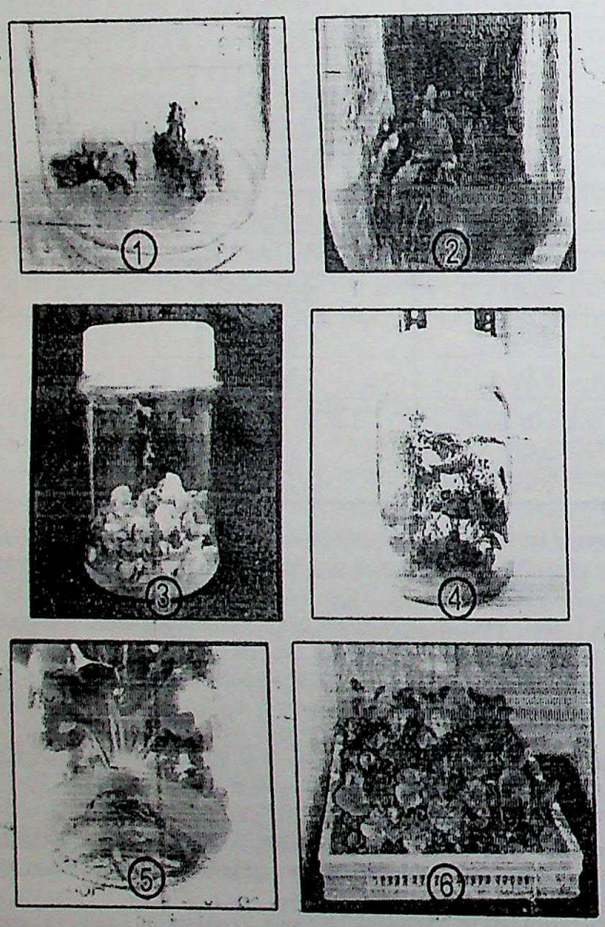

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Fig. 1. Callus induction on MS. medium supplemented with 1mg /l 2,4-D.
2. Callii differentiating into roots on MS medium supplemented with 2 mg/l NAA.
3. Shoot proliferation on MS + 0.5 mg/l BAP + 0.5 mg/l kinetin.
4. Shoot multiplication with 1.0 mg/l BAP + 1.0 mg/l kinetin.
5. Rooting of shoots with 1.5 mg/l IAA.
6. 21 days old gerbera plants in hardening unit.

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Influence of Organic Amendments on the Yield & Biological Efficiency of *Pleurotus sajor-caju* (Fr.) Singer

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The organic amendments *viz.*, neem cake powder, groundnut cake powder and rice bran were added at 5% and 10% levels to wet weight of the substrate into the mushroom bags as amendments during cultivation to study their effect on yield performance of *Pleurotus sajor-caju* (Fr.)Singer. Neem cake powder at 10% level significantly increased the sporophore production (980.0 gm) followed by 5% level (934.0 gm). All the bags took equal time for completion of spawn run *i.e.*, 17 days. Except neem cake powder and rice bran, all the other amendments had little effect on increasing the yield of *Pleurotus sajor-caju* (Fr.) Singer.

Keywords : Weed extract, biological efficiency and *Pleurotus florida*

INTRODUCTION

The cultivation of edible fungi has been accepted as the easiest on farm biotechnology for profitable removal of various lignocellulosic agricultural, industrial and forestry by-products, specially in the developing countries. Although about 200 species of edible fungi have been reported from India, only three *viz.*, *Pleurotus* spp., *Agraricus bisporus* and *Volvariella* species are preferred for commercial cultivation. *Pleurotus* species constitutes about 65% of the total mushrooms production in the country (Munjal, 1982). These species of mushrooms belong to the class Basidiomycotina and exhibit two distinct phases in their life cycle, vegetative phase called hyphae which branch and extend into mycelium and a reproductive phase characterized by the development of a fleshy sporophore referred to as mushroom fruit body. It is this structure, which is prized for its high nutritive value and culinary properties (Mehta, 1990).

Mushrooms are used as delicious flavored food and having nutritional value between meat and vegetables. Mushrooms are rich in protein, vitamin and minerals. It is low caloric food with very high potassium, sodium ratio and without starch as well as cholesterol. Vast quantities of renewable lignocelluloses wastes are generated every year in developing countries like India with economics, which are basically agricultural. However mushroom species has been reported to grow and yield successfully on many plant wastes.

Pleurotus species shows much diversity in their adaptation to the varying agro-climatic condition and show difference in yield depending on the substrates and organic amendments used. In the present investigation an attempt has been made to find out the role of organic amendments in increasing the yield of *Pleurotus sajor-caju* (Fr.) Singer.

MATERIAL AND METHODS

This work was carried out in biotech lab training and demonstration centre, Ambikapur, Chhattisgarh during July 2008 to November 2008. Parental strains of *Pleurotus sajor-caju* were provided by Mushroom Biotechnology lab, Indira Gandhi Agriculture University, Raipur, Chhattisgarh. The cultures were maintained on malt extract agar medium with regular sub-cultures at monthly. Spawn was prepared on wheat grain following standard procedure (Upadhyay *et al.*, 1997). 15 days old spawn was used for experimentation.

Organic amendments such as Neem cake, groundnut cake and rice were used as substrates along with wheat straw to evaluate their effect on the cultivation of *Pleurotus sajor-caju* (Fr.) Singer. All the amendments were powdered and sterilized at one hour. Wheat straw was used as substrates for experiments; the substrate was chemically sterilized with formalin and Bavistin. Wheat straw was soaked in separate containers in mushroom bags were prepared following the polybag method (Baskaran *et al.*, 1978). The organic amendments of Neem cake powder, groundnut cake powder and rice bran were added at 5% and 10% level to wet weight of the substrate into the mushroom bags. The amendments were thoroughly mixed with of the wheat straw before spawning. The polythene bags were then prepared by layer spawning methods. Suitable control was also maintained.

The bags were incubates in cultivation room at 25-30°C for the spawn run. When mycelia had completely covered the

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Table 1 : Effect of organic amendments on the sporophore production of *Pleurotus sajor caju*.

Substrates	Organic amendments	SRT (days)	Pin Head Initiation (day)	Total yield (gm/ Kg.)	Yield Differences From Control (gm)	BE (%)
Wheat Straw	Groundnut cake powder-5%	17	20	812	812-700 = +112	81.2
	Groundnut cake powder-10%	17	20	790	790-600 = +190	79.0
Wheat Straw	Neem cake powder-5%	17	20	934	934-700 = +234	93.4
	Neem cake powder-10%	17	20	980	980-700 = +280	98.0
Wheat Straw	Rice bran-5%	17	20	895	895-700 = +195	89.0
	Rice bran-10%	17	20	914	914-700 = +214	91.0
Wheat Straw	Control	17	20	700	700-700 = 0.0	70.0

bags, the polythene covering were turned off and relative humidity was maintained between 85-95%. Various parameters of cultivation, *viz.*, spawn run time, pinhead initiation; yield and biological efficiency were separately recorded for every subsequent flush. The biological efficiency of mushroom was determined as percentage yield of fresh mushroom in relation to dry weight of the substrate as suggested by Chang and Miles (1989). Those organic amendments showed significant yield difference were considered for mushroom cultivation.

RESULTS AND DISCUSSION

The result on the effect of organic amendments of neem cake powder, groundnut cake powder and rice bran at two concentrations on sporophore production of *Pleurotus sajor caju* (Fr.) Singer are presented in table-1. Neem cake powder at 10% Level significantly increased the sporophore production (980.0 gram) followed by 5% level (934.0 gram).All the bags took equal time for completion of spawn run *i.e.*,17 days. They did not show variation in primordial development and day of harvest even after application of organic amendments. It was due to short time interval between exposure of bags and pinhead initiation.

It was observed maximum in the bags treated with Neem cake powder at 10% (980gm, 98.0%) followed by Neem cake powder at 5% (934.4%),Rice bran-10% (914gm,91.4%) and Rice bran-5% (895 gm, 89.5%) and groundnut cake powder-10% (790.0 gm, 79.0%). Groundnut cake powder-5% (812gm, 81.2%) compare with control (700gm., 70.0%). It was either due to action of C: N present in organic amendment or to obvious reasons.

The reason for the increase in the yield of mushroom was due to increase in the protein content of the substrates (Karthikeyan, 1999). The addition of amendments to the substrates influences the sporophore production of *Pleurotus* species (Janaik, 1974). Supplementation of wheat straw substrates with powdered Neem cake helped in booting the yield of *Pleurotus sajor-caju*. The nature and amount of nitrogen present in the substrate after amendment influenced the yield of mushrooms. Addition of organic source of nitrogen had little effect on the yield of *Pleurotus sajor-caju*, While inorganic sources of nitrogen had a negative (Sivaprakasam, 1980).

India has still to make a humble beginning on this front otherwise also India does not figure among the leading mushroom producing countries at the present juncture he will have to make special efforts to improve this situation in the coming years, so that the targets and goals identified for the 21st century could by achieved for the overall benefit of the country.

Mushroom cultivation in Indian after a sluggish start, has exhibited swift and phenomenal growth, both in horizontal spread as vertical growth. The research support to this non-traditional crop has more or less kept pace its development during the last 3 decades. (Pathak, Yadav & Gour 2000).

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In Vitro Clonal Propagation of *Tylophora indica* (L.)

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Rapid multiplication of *Tylophora indica*, a medicinal plant was achieved by culturing of nodal segments on Murashige and Skoog's (MS) medium supplemented with various concentration of hormones (IAA, NAA, IBA, 2,4-D & BAP), High frequency of callulogenetic responses were recorded on medium containing 2, 4-D (5mg/l). Maximum number of shoots were recorded on medium containing BAP (5mg/l). Enormous rate of shoot and root explants were achieved through the various factorial combination and concentration of IAA and BAP (0.5, 1,2,5 mg/1). Regenerated plants were acclimatized and successfully transferred under field conditions.

Keywords : *In vitro* clonal propagation, MS medium, Callulogenetic response.

INTRODUCTION

Tylophora indica (Asclepiadaceae) is also known as *Tylophora asthmatica* is an important medicinal plant found in throughout India. Srilanka, Burma and Southeast asia. The roots and leaves are sweet, acrid, aromatic, emetic, purgative and expectorant. 200-400 mg of dried leaves and 1 -2 ml of leaves tincture per day can be used to treat asthma (Bone, 1996). They also possess curing properties of bronchitis, whooping cough, dysentry and diarrhoea. The powdered leaves, stem and roots contains 0.2 - 0.3% alkaloids, Tylophorine and Tylophoridine (Gopalakrishnan *et al.*, 1979).

Plant biotechnology involves the large scale culture of isolated plant cells which induce them to synthesise the natural secondary metabolites. In the present investigation carried out to develop the tylophora clone through tissue culture technology.

MATERIAL AND METHODS

The plants were collected from the herbal garden of S.T.E.T. Women's College, Mannargudi. Leaf, stem and node explants were used for the present study. The explants were excised with the help of sterile forceps and blade and they were cut 0.5 - 1 cm sized segments.

Explants were surface sterilized with 70% alcohol and then 0.1 % mercuric chloride. After sterilization they were rinsed 2-3 times with sterilized distilled water.

Explants were transferred to Murashige and Skoog medium (MS) supplemented with various growth regulators (2, 4-D, IAA, BAP, NAA) in various concentrations (0.5, 1,2, 5 mg/l).

The media of the pH was adjusted to 5.5 - 5.8 and 8g/l of agar was used as a solidifying agent. Explants with MS medium were maintained perfectly sterilized conditions and were incubated at 25 ± 2ºC. The cultures were kept under the light intensity of 2000 Lux at the level of culture tubes, using white fluorescent lamps. Explants and calli were sub cultured every 5 - 6 weeks.

RESULTS AND DISCUSSION

Different explants like leaf, stem and node of *Tylophora indica* Linn were cultured on different concentration of hormones (IAA, NAA, IBA, 2, 4 - D & BAP). It was observed that only nodal explants showed growth response like enlargement and initiation of callus. Therefore, only nodal explants of *Tylophora indica* Linn were used throughout the present study. Young node of *Tylophora indica* has succulent glonish nature and contains more meristamatic tissue than any other explants. The node explants are more ideal for induction of callus and multiple shoots because of the presence of more meristatic cells in young tissue.

MS basal medium supplemented with various concentrations of plant growth regulators (2, 4 - D, IAA, BAP). The maximum growth in terms of fresh and dry weight was observed at 5mg/l and minimum 0.5 mg/l in 2, 4 D.

Lee stadelmann *et al.*, (1989) and Mroginshi *et al.*, (1981) also demonstrated in popular tissue culture that node explants higher regenerative capacity than any other organs. Node explants are more sensitive to 2, 4 - D when compared to IAA, IBA, NAA and BAP.

Multiple shoots were formed on nodal explants of *Tylophora indica* after 3 - 4 weeks of culture period. MS medium supplemented with IAA individually or combination with BAP

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Table 1 : Effect of auxins and cytokinin on the nodal explants of *Tylophora indica*.

S.No.	Hormones concentration IAA + BAP (mg/l)	Formation of callus (%)	Formation of Shoot (%)	Formation of Root (%)	Fresh Wt. (mg)	Dry. Wt. (mg)
1.	0.5 + 0.5	62	-	-	900 ± 45.9	50.1 ± 11.2
2.	0.5 + 1.0	80	-	-	1200 ± 88.2	66.6 ± 14.5
3.	0.5 + 2.0	58	-	-	700 ± 39.3	38.8 ± 18.5
4.	0.5 + 5.0	61	-	-	850 ± 46.2	47.2 ± 21.3
5.	1 + 0.5	36	45	-	230 ± 21.3	12.7 ± 1.9
6.	1.+ 1.0	38	-	-	600 ± 37.2	33.2 ± 9.2
7.	1 + 2.0	58	42	-	1008 ± 98.3	55.2 ± 12.4
8.	1 + 5.0	75	-	45	1009 ± 96.8	55.4 ± 12.2
9.	2 + 0.5	60	-	-	1003 ± 96.4	55.7 ± 13.1
10.	2 + 1.0	62	-	-	1520 ± 112.7	84.2 ± 21.6
11.	2 + 2.0	64	-	-	2010 ± 157.5	111.0 ± 27.3
12.	2 + 5.0	80	-	-	2507 ± 280.8	139.0 ± 32.6
13.	5 + 0.5	-	-	-	18.5 ± 4.1	3.2 ± 0.9
14.	5 + 1.0	-	-	-	18.9 ± 4.2	3.5 ± 0.8
15.	5 + 2.0	-	-	-	18.5 ± 3.9	3.2 ± 0.9
16.	5 + 5.0	-	-	-	18.0 ± 3.8	3.1 ± 0.8

Incubation period : 6 weeks
Initial Fresh weight : 18.7 ± 4.6 mg
Initial Dry weight : 3.5 ± 0.9 mg

induced shoot from the node explants of *Tylophora indica*. BAP induced shoots were strong and dark green nature. Lower concentration induced minimum height but higher concentration induced maximum height of shoots. Well strong green nature of shoots with few numbers of leaves were observed on the media containing 5mg/l of BAP.

The role of growth regulators in growth and morphogensis of plant tissue cultures *in vitro* was known after the pioneering work of Skoog and Miller (1957). Morphogenetic potential of tissue explants is also altered by genetic and physiological age of mother plant (Liu and Chen, 1976; Raju and Mann, 1970).

5mg/l of NAA were induced callus with roots from the node explants. Generally inoculated nodes were formed callus first and then lateral roots were formed from the developed callus. Well developed roots were formed from the different concentration and combinations of IAA 0.5 + BAP 0.5 mg/l, IAA + BAP 2 mg/l; IAA + BAP 5mg/l. Both shoots and roots were induced on media containing auxin and cytokinin in the ratio of 1 and more than 1 (Table -1). However former development of shoots and roots did not occur on the same media after subculture.

These combinations of both hormones were gave the same results in different - plants: *Eclipta prostrata* (Suresh Kumar *et al.*, 1998) *Wedellia chinensis* (Kamalam, 1999).

Shoots and roots formed directly from explants were originated from the cut ends of nodal explants which consists of vascular cambium. Recent work by Subhersan (1998) also revealed formation shoot bud from cut ends of stem of *Enicostemma axillare*.

The rooted plantlets were transferred to polycups containing vermiculate for hardening. They were transferred to earthen pots containing garden soil and maintained in the research field

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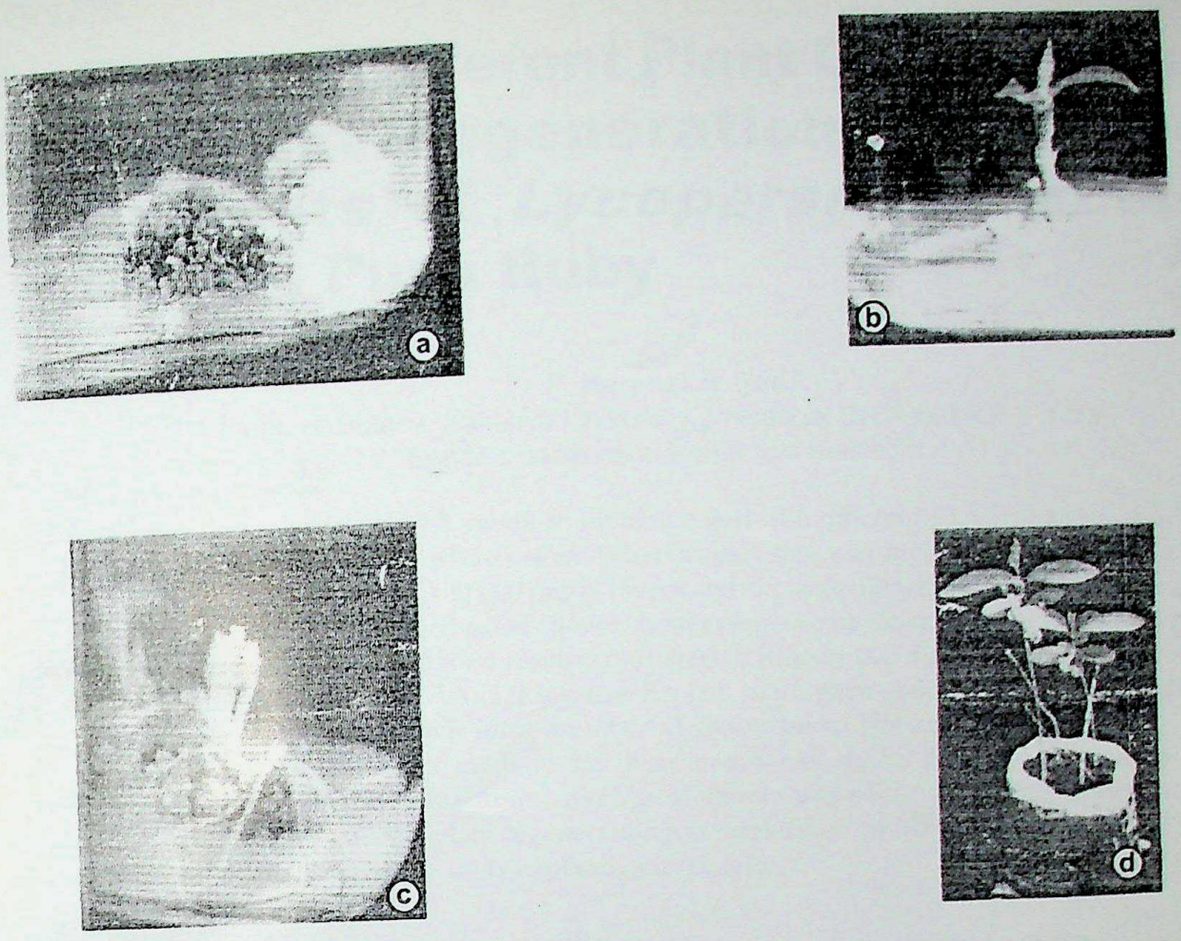

Fig - 1. Micropropagation of *T. indica*

a. Friable and brown colour callus from nodal explants on MS medium supplemented with 2, 4 - D, 5 mg/l.
b. Shoot induction from nodal explants on MS medium containing 5 mg/l BAP.
c. Well proliferated roots were initiated from the cut ends of node explants on callusing media with 1mg/l IAA and 2 mg/l BAP.
d. Successfully hardened plants of *Tylophora indica*

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Effect of Different Plant Growth Regulators on Plant Regeneration from Hypocotyl Explants of *Lycopersicon esculentum* Mill.cv. Pusa Ruby

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Hypocotyl explants cultured on M.S. medium supplemented with different IAA (1.0 mg/l), NAA (1.0 mg/l), 2, 4-D (1.0 mg/l) alone produced white callus. Whereas callusing was more pronounced in 1.0 mg/l IAA, compared to NAA (1.0 mg/l) and 2,4-D (1.0 mg/l). Hypocotyl explants cultured on M.S. medium supplemented with BAP (1.0 mg/l) or Kn (1.0 mg/l) alone showed better response in shoot differentiation with green callus. However, BAP (2.0 mg/l) showed good results compared to Kinetin (2.0 mg/l) in inducing shoot buds. IAA (1.0 mg/l) + BAP (2.0 mg/l) and IAA (1.0 mg/l) + Kn (2.0 mg/l) gave good response and produced shoots along with green nodular callus with more number of shoots buds. The combination of IAA (1.0 mg/l) + BAP (5.0 mg/l) found to be better medium for more number of shoots induction compared to all other concentrations of IAA + Kn. Maximum number of shoots was observed in the combination of IAA 1.0 mg/l + BAP 5.0 mg/l. Thus the plant regeneration was established on MS + 1.0 mg/l IAA + 2.0 mg/l BAP in tomato cv pusa ruby from hypocotyls explants, successfully.

INTRODUCTION

In Tomato (*Lycopersicon esculentum* Mill.), genetic transformation with regeneration *in vitro* has been successfully used for genetic improvement (Lindsey 1992). Regeneration of whole fertile plants from appropriate tissues *in vitro* is important in genetic plant transformation. Shoot formation from explants of apical meristems, cotyledons, hypocotyls, stems, petioles, leaves and inflorescence has been reported in tomato (Young *et al.*, 1987; Branca *et al.*, 1990; Compton and Veilleux' 1991, Sheeja *et al.*, 2004).

The most successful procedure for regeneration is through adventitious organogenesis (Van Rockel *et al.*,1993; Frary and Earle 1996; Peres *et al.*, 2001). *In vitro* plant regeneration has been found to depend on many factors of which most important are composition of the basic medium, growth regulators, light intensity and quality, photoperiod, temperature, cultivation vessels and vessels covers (Reed 1999). The purpose of our work is to determine the effect of different growth regulators on plant regeneration from hypocotyl explants of tomato.

MATERIAL AND METHODS

The seeds of *L. esculentum* c.v. Pusa Ruby obtained from Maharashtra Hybrid Seeds Co., were surface sterilized with 70% ethanol for 30 seconds followed by 0.2% mercuric chloride for 5 min following rinsing (several times) with distilled water. Seeds were germinated aseptically on MS basal medium, gelled with 0.8% agar and cultures were incubated at 25 + 2°C under 16 hrs photoperiod (30 μ mol $M^{-2}S^{-1}$). The Hypocotyl explants were excised from *in vitro* grown seedlings and cut into 0.5 - 1 cm in length and inoculated on M.S. medium augmented with different concentrations and combinations of auxins and cytokinins (Table 1 and 2). They were placed laterally, so that both the cut ends remained in contact with the medium.

RESULTS AND DISCUSSION

Hypocotyl explants of *L. esculentum* cv Pusa Ruby were cultured on MS medium containing IAA(1.0 mg/l), NAA (1.0 mg/l) and 2, 4-D(1.0 mg/l) alone produced whitish callus. Callusing was more pronounced in case of 1.0 mg/l IAA than NAA(1.0 mg/l). Whereas 2, 4-D(1.0 mg/l) showed less response. Hypocotyl explants cultured on MS medium supplemented with BAP(1.0 mg/l) or Kn (1.0 mg/l) alone showed better response in shoot differentiation with green callus. However, BAP(2.0 mg/l) showed good as compared to Kinetin (2.0 mg/l) in inducing shoot buds. Among the various combinations of auxins with cytokinins NAA (1.0 mg/l) in combination with BAP(2.0 mg/l) and IAA(1.0 mg/l) with Kinetin(2.0 mg/l) gave good response and produced green callus with more number of shoot buds. Percentage of responding cultures and more number of shoot buds were observed on IAA (1.0 mg/l) + BAP (5.0 mg/l) combination

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Table 1 : Effect of IAA,NAA,2,4-D,BAP, Kinetin alone and in combination on morphogenic response of hypocotyl culture of *L. esculentum* cv. Pusa Ruby.

Growth regulators (mg/l)	% of response	Morphogenic response
IAA 1.0	45	Callus
NAA 1.0	42	Callus
2, 4-D 1.0	38	Callus
BAP 1.0	48	Green callus + shoots
Kinetin 1.0	38	Green callus + shoots
IAA 1.0 + BAP 0.5	37	Callus + Shoots
IAA 1.0 + BAP 1.0	45	Green nodular callus + shoots
IAA 1.0 + BAP 2.0	58	Green nodular callus + shoots
IAA 1.0 + Kinetin 0.5	36	Callus + Shoots
IAA 1.0 + Kinetin 1.0	43	Callus + Shoots
IAA 1.0 + Kinetin 2.0	50	Callus + Shoots
IAA 1.0 + BAP 0.5	50	Nodular Callus
NAA 1.0 + BAP 1.0	62	Nodular Callus
NAA 1.0 + BAP 2.0	70	Nodular Callus
NAA 1.0 + Kinetin 0.5	58	Nodular Callus
NAA 1.0 + Kinetin 1.0	61	Nodular Callus
NAA 1.0 + Kinetin 2.0	67	Nodular Callus

Data scored after six weeks of culture based on 10 replicates.

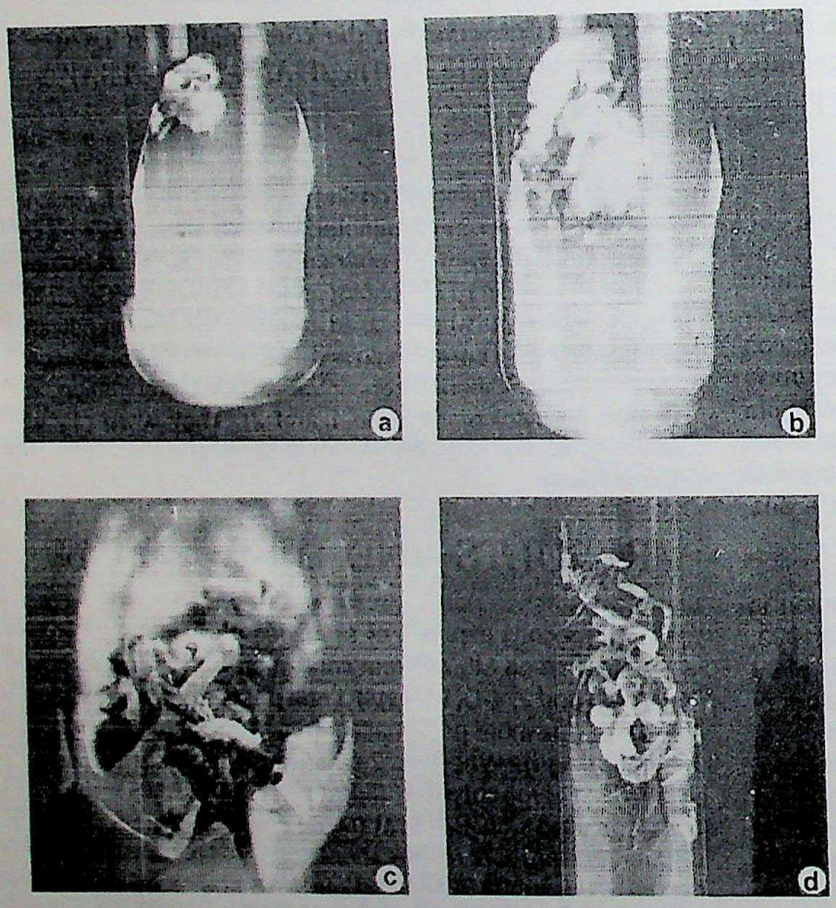

Figure Legends :

Fig. 1 : Plantlet regeneration from hypocotyl cultures.

a. Callus induction on MS+1.0 mg/l IAA

b. Callus induction on MS+1.0 mg/l NAA

c. Multiple shoots induction on MS + 1.0 mg/l IAA + 2.0 mg/l BAP

d. *In vitro* rooting on MS + 1.0 mg/l IAA

compared to IAA (1.0 mg/l) + Kinetin (5.0 mg/l). Data on shoot buds formation in hypocotyl cultures showed high percentage of response with maximum number of shoots per explant compared to various concentrations of IAA and Kn combination. More number of shoots (6.2 ± 0.24) was observe on 1:0 mg/l IAA + 5.0 mg/l BAP (Table 1 & 2).

Jawahar *et al.* (1997) have studied the *in vitro* hypocotyl callus cultures and plant regeneration of tomato cv PKMI hypocotyl explants and observed frequency of callus induction increased with increasing concentrations of auxins and the optimal levels being 2 mg/l IAA and 2 mg/l NAA in addition to the BA and Kinetin. From results of present study it is evident that MS + 1.0 mg/l IAA + 2.0 mg/l BAP was found to be best in obtaining hypocotyl cultures in tomato cv pusa ruby.

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Table 2 : Effect of IAA in combination of BAP and Kinetin on plant regeneration in Hypocotyl cultures of *L. esculentum* cv. Pusa Ruby

Growth hormones (mg/l)			Morphogenic response	
IAA	BAP	Kinetin	% of cultures with shoots	Mean No. of sheets per explant
0.5	1.0	--	46	1.2 ± 0.01
0.5	1.5	--	45	1.0 ± 0.12
0.5	2.0	--	47	1.3 ± 0.22
0.5	2.5	--	41	2.1 ± 0.12
0.5	3.0	--	38	2.2 ± 0.32
0.5	3.5	--	47	3.1 ± 0.34
0.5	4.0	--	41	3.2 ± 0.21
0.5	4.5	--	50	3.3 ± 0.51
0.5	5.0	--	69	4.6 ± 0.21
1.0	0.5	--	36	0.1 ± 0.12
1.0	1.0	--	40	1.1 ± 0.22
1.0	1.5	--	42	1.6 ± 0.18
1.0	2.0	--	49	2.2 ± 0.32
1.0	2.5	--	50	2.5 ± 0.12
1.0	3.0	--	51	2.6 ± 0.11
1.0	3.5	--	61	3.1 ± 0.12
1.0	1.0	--	62	3.8 ± 0.29
1.0	4.5	--	61	4.7 ± 0.31
1.0	5.0	--	75	6.2 ± 0.24
0.5	--	1.0	60	0.5 ± 0.21
0.5	--	1.5	61	0.8 ± 0.32
0.5	--	2.0	50	1.0 ± 0.12
0.5	--	2.5	51	1.2 ± 0.23
1.0	--	0.5	35	0.3 ± 0.16
1.0	--	1.0	44	1.0 ± 0.32
1.0	--	1.5	49	1.1 ± 0.12
1.0	--	2.0	51	1.2 ± 0.13
1.0	--	2.5	55	1.8 ± 0.12

Data scored after six weeks of culture based on 10 replicates.

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Comparison Between Antioxidant Activity of two Medicinal Plants belong to Family Acanthaceae

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Recent investigations have shown that the antioxidant potential of plants could be correlated with oxidative strees defence and different human diseases. In this respect Flavonoids and other polyphenolic compounds have gained the greatest interest. The present study was undertaken to evaluate the *in vitro* antioxidant activities of aqueous as well as methanolic extract of *Andrographis paniculata* and *Justicia adhatoda*, belongs to the same family named Acanthaceae.

The 10% aqueous and methanolic extract of each plant part (root, stem & leaf) was prepared separately and were tested for DPPH (2,2 - diphenyl, 2 - picryl hydrazyl) radical scavenging, nitric oxide radical scavenging and flavonoid content. The results obtained in the present study indicate that *Justicia adhatoda* is a potential source of natural antioxidants.

Keywords : DPPH, Nitric oxide (NO), *Andrographis paniculata* (Burm. F.) Nees, *Justicia adhatoda* (L.) etc.

INTRODUCTION

Medicinal plants are a major source of drugs for the treatment of various health disorders all over the World. The plant materials contain thousands of chemicals which act against diseases and infections of humans and animals, when properly used. According to assessment of WHO about 80% of World population depend on medicinal based on plants (Shinwari & Khan, 1998).

Andrographis paniculata (Burm F.) and *Justicia adhatoda* (L) are the most important plant species and both are belong to family Acanthaceae. They are evergreen gregarious shrubs. Besides various medicinal properly they share a common property *i.e.*, anti inflammatory property.

MATERIAL AND METHODS

Various anti oxidant activity methods have been used to monitor and compare the anti oxidant activity of medicinal plants. Free radicals are involved in many disorders like - neurodegenerative diseases, cancer and AIDS. Anti oxidants through their scavenging power are useful for the management of those diseases.

There are various methods which can be employed in evaluation of anti oxidant activity *in vitro*, for instance - DPPH (2,2 - diphenyl,2-picryl hydrazyl) radical, Nitric oxide (NO) radical etc scavenging assay.

In order to calculate anti oxidant activity, 10% aqueous as well as alcoholic extract was prepared for each plant part (root, stem & leaf), separately.

1- DPPH assary - (By Brand- Williams, Cuvelier & Berset; 1995)

This assay is based on scavenging on DPPH *i.e.*, 2, 2-diphenyl,2-picryl hydrazyl from the anti oxidants which produces a decrease in absorbance at 517 nm, When solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by less of colour.

Each herbal extract was added at an equal volume, to Methanolic solution of DPPH (0.1mM). The contents were mixed property and incubated at room temperature (25°C) for 15 minutes. After 15 min. at room temperature, the absorbance was recorded at 517 nm.

Vitamin P (rutin) was used as reference compound.

"Low absorbance indicates high free radical scavenging activity"

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbacne of sample} \times 100}{\text{Absorbance of control}}$$

2- Nitric oxide (NO) radical inhibition assay - (By Green L.C., *et al;* 1982). This assay is based on the method, where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using

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Griess reagent (1% sulphanilamide, 2% ortho phosphoric acid, 0.1% naphthylene diamine hydrochloride). Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions.

Herbal extracts were added in an aliquot followed by adding one ml of buffer and one ml of sodium nitroprusside (10mM). Now the tubes were incubated at 25ºC for 150 min. One ml of incubated solution was added in one ml of Griess reagent.

Pink coloured chromophore was observed under diffused light. Then absorbance was measured at 546 nm.

Vitamin P (rutin) was used as reference compound.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbacne of sample} \times 100}{\text{Absorbance of control}}$$

3 - Determination of Flavonoid content - Flavonoids were determined by a Pharmacopeia method (State Pharmacopeia of USSR, 1998), using rutin or vitamin P as reference compound.

Herbal extracts were mixed with two ml of aluminium chloride (2%), followed by incubation at 20ºC for 30 min. Absorbance was measured at 430 nm.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbacne of sample} \times 100}{\text{Absorbance of control}}$$

RESULTS AND DISCUSSION

Results of the comparison between the per cent inhibition of antioxidant activity of *Andrographis paniculata* and *Justicia adhatoda* are given in the Table 1-2. As the DPPH results are concerned the low absorbance indicates the high free radical scavenging activity, thus methanolic solution of stem of *J. adhatoda* showed maximum free radical scavenging activity whereas minimum free radical scavenging activity was showed by aqueous solution of stem of *A. paniculata*.

Table 1 : Per cent inhibition of anti oxidant present in *Andrographis paniculata*.

S.No.	Anti oxidant Assay	Root		Stem		Leaf	
		Aq.	Alc.	Aq.	Alc.	Aq.	Alc.
1.	DPPH Assay	8.82%	14.31%	5.45%	14.41%	14.08%	19.86%
2.	Nitric Oxide Assay	47.44%	67.11%	63.49%	38.47%	57.82%	88.59%
3.	Flavonoid Assay	63.74%	74.59%	89.53%	67.59%	65.74%	15.93%

Table 2 : Per cent inhibition of anti oxidant present in *Justicia adhatoda*.

S.No.	Anti oxidant Assay	Root		Stem		Leaf	
		Aq.	Alc.	Aq.	Alc.	Aq.	Alc.
1.	DPPH Assay	6.01%	97.87%	14.64%	97.90%	6.40%	22.54%
2.	Nitric Oxide Assay	31.54%	63.72%	73.40%	54.68%	41.46%	53.10%
3.	Flavonoid Assay	93.53%	98.61%	98.69%	97.84%	46.03%	0.15%

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radicals can bring out thousands of reactions and thus may cause extensive tissue damage. Antioxidants may offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid per oxidation etc.

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc, and involved in the regulation of various physiological processes. Excess concentration of nitric oxide is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals. In the present study, the extract competes with oxygen to react with nitric oxide and thus inhibits the generation of anions. Thus, results revealed that maximum per cent inhibition was showed by the methanolic extract of leaf of *A. paniculata* whereas minimum per cent inhibition was showed by aqueous extract of root of *J. adhatoda*.

Flavonoids are groups of naturally occurring compounds widely distributed, as secondary metabolites in the plant kingdom. The Flavonoids have also been reported to possess antioxidant and antiradical properties (Nakayoma and Yamada 1995). Maximum flavonoid content was reported in the aqueous extract of stem of *J. adhatoda* whereas minimum flavonoid content was observed in the methanolic extract of leaf of the same plant.

This study suggested that the *J. adhatoda* extract possesses antioxidant activity, which might be helpful in preventing or slowing the process of various oxidative stress related diseases.

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Efficacy of Local Isolates of *Trichoderma* spp. Against *Fusarium oxysporum* f.sp. *Carthami* Causing Wilt of Safflower

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In present study, fifty promising isolates of *Trichoderma* spp. were obtained using TSMC. Efficacy of *Trichoderma* isolates against *F. oxysproum* f.sp. *Carthami* was tested in laboratory by dual culture technique under *in vitro* conditions. Results indicated that in general *Trichoderma* isolates inhibited growth of the pathogen. At 9 days after incubation, minimum growth of the pathogen was observed in isolate 29 followed by isolate 33 *Trichoderma* isolates 20, 40, 19, 17, 48, 10, 12, 2, 47 and 8 were fast growing as compared to other *Trichoderma* isolates taken in the study.

Keywords : *F. oxysporum* f.sp. *Carthami, Trichoderma* spp., Dual culture, Inhibition of growth.

INTRODUCTION

Wilt of safflower caused by *Fusarium oxysporum* f.sp. *Carthami* is one of the serious diseases of the crop. Since the disease is soil and seed borne, is difficult to manage. Only practical solution seems to be use of resistant variety. However, resistant varieties for this disease are not available. In this situation, farmers are facing huge loss and in many parts have opted for cultivation of other crops. Present study was aimed to evaluate local isolates of *Trichoderma* spp. against the pathogen. Since bio-control agents are eco-friendly, cheap and cost effective can be one of the component of integrated disease management strategy.

MATERIAL AND METHODS

Rhizosphere samples from various crops grown in eight districts of Marathwada region and Malegaon of Nashik district were collected. Isolations of *Trichoderma* spp. were carried out on *Trichoderma* selective medium C without quintozone (PCNB) under aseptic condition. The plates were incubated in the laboratory at 25 ± 1°C. The isolated fungi were purified by hyphal tip method after growing on petridishes. The isolated fungal organisms were identified upto generic level based on colony characters, growth and morphology of the fungus. For this morphological descriptions were compared with the standard descriptions made in text (Nagmani, 2002) for confirmation.

Efficacy of fifty promising true to type isolates of *Trichoderma* spp. was tested against *F. oxysporum* f.sp. *Carthami* in dual culture. For this, *F. oxysporum* f.sp. *Carthami* and *Trichoderma* isolates were multiplied on sterilized PDA medium in petri plates for a weak's period. Growth of the pathogen (5 mm disc) was taken out with the help of cork borer and was placed at one end of the plate. Just opposite to it growth (5 mm disc) of respective *Trichoderma* spp. was placed. Control plate consisted of growth of pathogen only. There were two replications. Plates were incubated at 25 ± 1°C and colony diameter of the pathogen and *Trichoderma* spp. were recorded at 9 days after incubation.

RESULTS AND DISCUSSION

Perusal of results from Table 1 indicated that growth of *Fusarium oxysporum* f.sp. *Carthami* was significantly influenced by various isolates of *Trichoderma* spp. under study at 9 days after incubation.

At 9 days after incubation, except *Trichoderma* isolates 8, 1,10, 17, 31, 5, 36, 22, 20 and 35 all the other *Trichoderma* isolates significantly reduced growth of the pathogen as compared to control. Minimum growth of the pathogen was observed in isolate 29 which was at par with isolates 33, 32, 24, 3, 9, 16, 26, 50, 39, 12, 19, 46, 15, 14 and 38 significantly less than rest of the isolates. It was followed by isolate 33 which was at par with all the aforesaid isolates and isolate 40 and significantly less than growth in the remaining isolates. Besides these two isolates other *Trichoderma* isolates restricting growth of the pathogen below 10 mm include isolates 32, 24 and 3. The isolates restricting growth of the pathogen between 10 and 20 mm include isolates 9, 16, 26, 50 and 39. *Trichoderma* isolates restricting growth of the pathogen between 20 and 30 mm, include isolates 12, 19, 46,15,14,38, 40, 44, 41, 2, 13, 49 and 45.

At 9 days after incubation, maximum growth was observed in isolate 20 which was at par with isolates 40, 19,

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Table 1 : Influenced *Trichoderma* isolates on growth of *Fusarium oxysporum* f.sp. *Carthami*.

Trichoderma isolates No.	Growth of *Fusarium oxysporum* f.sp. *Carthami* (9 DAI)	Growth of *Trichoderma* isolate (9 DAI)
1	42.60	29.50
2	28.75	56.80
3	9.7	30.50
4	41.30	49.45
5	47.30	51.95
6	28.60	23.70
7.	34.25	27.35
8	41.95	56.10
9	10.10	37.00
10	42.70	64.65
11	33.00	39.80
12	20.10	59.30
13	29.60	37.75
14	24.30	40.65
15	23.30	41.10
16	13.80	40.15
17	43.85	66.45
18	21.95	34.80
19	55.00	67.50
20	39.55	76.25
21	38.70	48.80
22	54.55	22.15
23	31.95	18.95
24	9.5	42.80
25	34.00	44.15
26	16.70	40.0
27	36.35	29.00
28	36.05	51.50
29	6.20	33.10
30	6.95	26.50
31	45.85	53.55
32	8.60	29.0
33	7.2	21.05
34	34.95	31.30
35	61.45	38.50
36	50.30	46.75
37	39.50	39.10
38	25.95	51.00
39	18.95	38.30
40	26.65	69.0
41	27.50	47.60
42	28.80	36.00
43	33.50	32.60
44	27.20	26.65
45	29.95	31.30
46	22.50	54.10
47	38.70	56.20
48	30.55	64.80
49	29.70	44.50
50	17.80	31.45
51	61.45	
SE ±	7.190	7.69
CD at 5%	19.89	21.30

DAI : Days after incubation.

17, 48, 10, 12, 2, 47 and 8 and significantly higher than rest of the isolates. *Trichoderma* isolates having growth between 60 and 70 mm include isolates 10, 48, 17, 19 and 40. Isolates having growth between 50 and 60 mm include isolates 38, 28, 5, 31; 46, 8, 47, 2 and 12.

Several workers in past reported efficacy of *Trichoderma* spp. especially, *Trichoderma virtde* and *T. harzianum* against this pathogen (Anonymous, 1998; Chattopadhay and Shastri, 1999 and 2001; Behere, 2002 and Prasad, 2003). These observations support finding of present study.

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Comparative Antibacterial Efficacy of Methanol and Aqueous Extracts of *Calycopteries floribunda* (Roxb.) Poiret

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Calycopteries floribunda (Roxb.) Poiret (Combretaceae) commonly grows in semi-evergreen forests of Western Ghats. The present study was undertaken to investigate the phytoconstituents and antibacterial activity of methanol and aqueous extracts of leaves of *C. floribunda*. The powdered plant material was subjected to extraction using methanol and water as solvents. The extracts were screened for antibacterial activity by Agar-well-diffusion method. In the preliminary phytochemical analysis, the methanol extract revealed the presence of alkaloids, tannins, flavonoids, saponins and terpenoids. Among extracts tested, methanol extract showed more inhibition of test bacteria than aqueous extract. Gram-positive bacteria were more inhibited than Gram-negative bacteria. The antibacterial activity of solvent extracts may be due to the presence of various phytoconstituents. Further studies are to be carried to isolate active constituents and reveal the efficacy of extracts *in vivo*.

Keywords : *Calycopteries floribunda* (Roxb.) Poiret, Phytochemical screening, Antibacterial activity.

INTRODUCTION

It is estimated that there are 250,000 to 500,000 species of plants on earth (Barris, 1996). Relatively small percentages (1 to 10%) of these are used as foods by both humans and other animal species. It is possible that many more are used for medicinal purposes (Moerman, 1996). The use of plant extracts to treat infections is an age-old practice in a large part of the world, especially in developing countries, where there is dependance of traditional medicine for a variety of diseases (Shiba *et al.*, 2005). Interest in plants with antimicrobial properties has been revived as a result of current problems associated with the use of antibiotics (Abu-Shanab *et al.*, 2004). Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982). A vast knowledge of medicinal plants has accumulated in various regions (Diallo *et al.*, 1999). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds (Edeoga *et al.*, 2005). *Calycopteries floribunda* (Roxb.) Poiret (Combretaceae), locally called Marasuttu balli, is a straggling shrub with leaves opposite, acuminate round at the base, with sub-terminal branched raceme inflorescence, calyx with five angled lobes acrescent with ten stamens, fruit ovoid, five angled and narrow. Flowering and fruiting occurs between January to April. The plant commonly grows in semi-evergreen forests of Western Ghats. The leaves are bitter, astringent, antihelmintic, antiemetic and are used in colic (Balakrishna Gowda, 2004). The present study aims to screen the presence of various phytoconstituents and antibacterial activity of methanol and aqueous extracts of *C. floribunda*.

MATERIAL AND METHODS

Collection and identification : The plant material was collected in Hosanagara, Shimoga district during November 2008 and authenticated to identity in the Department of Applied Botany, Jnanasahyadri, Kuvempu University, Shankaraghatta, Karnataka. Voucher specimen (KU/AB/KSV/212) was deposited in the department for future reference.

Extraction and phytochemical analysis : The plant material was washed thoroughly with running tap water, shade dried, powdered and used for extraction. A known amount of powdered material (500gm) was subjected to soxhlet extraction and exhaustively extracted with methanol for about 48 hours. The extract was filtered and concentrated in vacuum under reduced pressure using rotary-flash evaporator and dried in the desiccator (Manjunatha *et al.*, 2006). Methanolic extract was subjected to preliminary phytochemical screening to screen the presence of various secondary metabolites in the solvent extracts (Parekh and Chanda, 2007).

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Hot water extraction : Ten gram of plant material was boiled in 100ml of distilled water with constant stirring for about an hour. The solution was allowed to attain room temperature and filtered through three-fold muslin cloth followed by Whatman filter paper No. 1. The filtered extract was evaporated, condensed and used for antibacterial studies (Beltrame *et al.*, 2002).

Antibacterial activity of methanol and aqueous extract : The efficacy of methanol extract of *C. floribunda* was tested against Gram positive bacteria- *Staphylococcus aureus* MTCC-902 and *Clostridium perfringens* MTCC-450 and Gram negative bacteria *Escherichia coli* MTCC-405, *Klebsiella pneumoniae* MTCC-432, *Salmonella typhimurium* MTCC-1252 and *Pseudomonas aeruginosa* MTCC-1934 by Agar-well-diffusion method (Adeniyi and Ayepola, 2008). The bacteria were procured from Institute of Microbial Technology (IMTECH), Chandigarh. In this method, 24 hours-old Muller-Hinton broth cultures of test bacteria were swabbed uniformly on solidified sterile Muller-Hinton agar plates using sterile cotton swab. Then, aseptically wells of 6mm diameter were bored in the inoculated plates with the help of gel puncher and the extract (10mg/ml of DMSO), Standard (Chloramphenicol, 1mg/ml) and Control (10% DMSO) were added into the respectively labeled wells. The plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition was recorded. The experiment was carried in triplicates to get average reading.

RESULTS AND DISCUSSION

The preliminary phytochemical tests showed the presence of tannins, terpenoids, alkaloids, saponins, steroids and flavonoids in the solvent extract. The result of antibacterial activity of methanol extract of *C. floribunda* is given in Table-1. Results were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and it as reported as positive and absence of zone as negative (Panthi and Chaudhury, 2006). It is clear that the methanol extract, aqueous extract and the standard antibiotic were more active against Gram-positive bacteria than against Gram-negative bacteria. The greater resistance of Gram-negative bacteria to plant extracts has previously been documented and related to thick murein layer in their outer membrane, which prevents the entry of inhibitor substances into the cell (Palombo and Semple, 2001). Among Gram-negative bacteria tested, methanol extract caused greater inhibition of *E. coli* (ZOI 14mm) followed by *K. pneumoniae* (11mm), *S. typhimurium* (20mm) and *P. aeruginosa* (09mm). In case of Gram-positive bacteria tested, *S.aureus* was relatively affected more (16mm) than *C. perfringens* (14mm). The aqueous extract exhibited less antibacterial activity as compared to methanol extract except in case of *K. pneumoniae* and *C. perfringens* where the activity was found to be same as that of methanol extract. Among Gram-negative bacteria, *P. aeruginosa* was least affected by extracts, while *E. coli* was found to be more susceptible.

Table 1 : Antibacterial activity of methanol extract of *C. floribunda*.

Bacteria	Zone of inhibition (ZOI) in mm			
	Methanol extract	Aqueous extract	Standard	Control
E. coli	14	12	22	-
S. typhimurium	10	09	20	-
K. pneumoniae	11	11	20	-
P. aeruginosa	09	08	18	-
C. perfringens	14	14	26	-
S. aureus	16	15	24	-

Results are average of three trials

In developing countries like India knowledge of plant derived metabolites could reduce the cost of healthcare. India has a rich history of using various herbs and herbal components for treating various diseases (Ali *et al.*, 2008). Antimicrobial activity of tannins (Ho *et al.*, 2001; Doss *et al.*, 2009), flavonoids (Pepeljnjak *et al.*, 2005; Mandalari *et al.*, 2007), saponins (Baharaminejad *et al.*, 2007; Avato *et al.*, 2006), terpenoids (Funatogawa *et al.*, 2004), alkaloids (Navarro and Delgado, 1999; Faizi *et al.*, 2003) have been documented.

Calycopteris floribunda is a large climbing woody shrub and is well distributed south-east Asian countries. Traditionally, *C. floribunda* has been used in colic, as an antihelminthic, astringent and carminative, and for the treatment of diarrhoea, dysentery, jaundice and malaria in many countries (Ali *et al.*, 2008). The aqueous 90% methanol and 1-butanol soluble fractions of the leaves showed significant antioxidant activity. Two pure compounds, 3,8-di-O-methyl ellagic and 2,3,7-tri-O-methyl ellagic acids were isolated from 1-butanol soluble fraction of the mother extract (Dey *et al.*, 2005). Antibacterial and antioxidant efficacy of Dichloromethane-methanol extract of leaves of *Calycopteris floribunda* and antibacterial activity of aqueous 90% methanol soluble fractions was shown against *B. subtilis, S. pyogenes, S. aureus* and *S. typhi* (Dey *et al.*, 2005). Mayer (1999) isolated neocalycopterone along with two new biflavonoids, calyflorenones A and B from dried leaves of *C. floribunda*. Ali *et al.*, (2008) isc'ated pachypodol, a flavonol from the leaves of *C. floribunda* that inhibited the growth of Cac(O) 2 colon cancer cell line *in vitro*. Three new biflavonoids calycopterone, isocalycopterone, and 4 demethylcalycopterone and the known flavone 4',5-dihydroxy-3,3',6,7 tetramethoxyflavone were isolated as cytotoxic constituents from the flowers of *C. floribunda* Lamk. Compounds showed a wide range of activity against a panel of solid tumor cell lines. Among the biflavonoids, calycopterone is the major constituent (Wall *et al.*, 1994). The structures of five biflavonoids, 6"-demethoxyneocalycopterone, calyflorenone C, 6"-*epi*-calyflorenone B, 6"-*epi*-calyflorenone C and calyflorenone D from the green leaves of *C. floribunda* were established by NMR and MS (Mayer, 2004).

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. In recent years development of multidrug resistance in the pathogenic bacteria and parasites has created major clinical problems in the

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treatment of infectious diseases (Davies, 1994) and other problems such as toxicity of certain antimicrobial drugs on the host tissue (Idose *et al.*, 1968; Maddux and Barrere, 1980) triggered interest in search of new antimicrobial substances/ drugs of plant origin. Phytomedicines have shown great promise in the treatment of various diseases including viral infections. Single and polyherbal preparations have been used throughout human history for the treatment of various types of illness (Adwan, 2006). Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extracts for their antimicrobial activity may provide new anti-microbial substances. The results of this study are promising against the bacteria tested. As the study made use of bacteria which are known to cause various types of illness such as food poisoning, hospital infections, enteric infections etc, the extracts could be used to treat infections caused by these pathogenic bacteria.

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Studies on the Antibacterial, Antifungal and Antioxident Activities of *Saussurea costus* - A Critically Endangered Medicinal Plant of N.W. Himalaya

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The therapeutic potentials of *Saussurea costus*, an important medicinal herb traditionally used in China to treat gastrointestinal infections, was evaluated for its antibacterial, antifungal and antioxidant activities. The methanolic, ethanolic, chloroform, isopropanolic and aqueous root extracts of *Saussurea* were first investigated against mixed culture of bacteria and fungi grown on the specific medium and antioxidant activity was checked for methanol extract only. Among various extracts, the maximum activity was exhibited by methanolic extract. By various assays like DPPH assay, TAB asaay, LPA on PMS and microsomes and NBT assay, it was established that the alcoholic extracts of *Saussurea costus* possessed antioxidant activity.

Keywords : Medicinal plants, antibacterial activity, aqueous extract, methanol extract.

INTRODUCTION

Plants still constitute one of the major source of raw materials for manufacture of drugs for treating various ailments of human beings. Although there hss been significant development in the field of synthetic drug chemistry and antibiotics. The awareness of the wide spread toxicity and harmful after effects associated with their long use, the societies prefer drugs from natural sources than synthetic ones. A large variety of herbal drugs mentioned in India system of medicine for their antibacterial, antifungal and antioxidant properties have been under study. Antimicrobial agents can be derived from herbs, as over 1000 plants exhibit antimicrobial effects (Nyches, 1975; Kambu *et al.*, 1990; Kaushik, 2003; Sumitra *et al.*, 2006; Mi-Hyang Lee *et al.*, 2006 and Jigna and Sumitra, 2007). These plants are believed to promote positive health and maintain a nice physique, besides maintaining organic resistance against infections by re-establishing body equilibrium and conditioning the body tissues (Bagwandas, 1978). Hence, the drugs of plant origin are gaining tremendous popularity and are being investigated for correction of various pathological conditons.

Medicinal herbs are being studied increasingly by pharmacological researchers, and many such herbs have a long history of medicinal use in Asia (Sinclair, 1998). These herbs have many potential clinical and therapeutic applications in the modern medical setting, as numerous studies have revealed that they contain bioactive components, and have resulted in a better understanding of their physiological, therapeutic and clinical actions (Merken *et al.*, 2001; Zheng and Wang, 2001). Traditionally, these herbs are said to provide safe and effective treatments against many diseases. Recent work has revealed the potential of several herbs as sources of drugs (Iwu, 2002). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plant represent a potential source of novel antibiotic prototypes (Afolayan, 2003).

Oxygen derived free radicals, such as the superoxide anion and hydroxyl radical, are cytotoxic and promote tissue injury (Peterhans, 1997). Antioxidants act as a major defense against radical mediated toxicity by protecting the damage caused by free radicals (Nayan Jose and Janardhanan, 2000). Furthermore although medicinal plants are used as 'antioxidants' in traditional medicine, their claimed therapeutic properties could be due.,in part, to their capacity for scavenging oxygen free radicals. Many plants contain substantial amounts of antioxidants including Vitamin C and E, carotenoids, flavonoids, tannins and thus can be utilized to scavenge the excess free radicals from the human body. Epidemiological studies have suggested the association between the consumption of antioxidant rich foods and beverages and the prevention of diseases. There is a lot of on going research on such plants for their potential usefulness as dietary supplements and as adjuvants for use in therapeutic management of stress related disorders.

Saussurea costus (Falc.) Lipsch, commonly called as kuth an alpine herb distributed in N,W. Himalaya, is one such plant which has been traditionally used in treatment of cough

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and asthma wherein alcoholic extract is useful in treatment of vagotonic type bronchial asthma (Koul, 1997). The root extract is an important source of an alkaloid saussurine which is traditionally used for treatment of stomach cramps, dysentery and joint pains. The roots are also used medicinally as tonic, carminative, stimulant and in treatment of bronchial asthma Considerable antifungal activity has also been reported against pathogenic fungi (Gupta and Singh, 1982; Singh *et al.*, 1983). The antioxidant activity of *Saussurea costus* has also been reported (Pandey *et al.*, 2005). The need of the hour is to screen a number of medicinal plants for promising biological activity. In the present work extracts of *Saussurea costus* a medicinal plant was evaluated for its antibacterial, antifungal and antioxidant properties, Since little experimental evidence is available about medicinal role of *Saussurea costus* of N.W. Himalayas.

MATERIAL AND METHODS

Plant Material : The plant material was collected from its natural habitat in Kashmir Himalaya and identified in the Department of Botany (centre of plant taxonomy), University of Kashmir, Srinagar. The root of *Saussurea costus* family (Asteraceae) were cut from the plant thoroughly washed with tap water, rinsed three to four times with distilled water and then dried in shade. The dried roots were cut into small pieces, powdered in a grinder and then extracted with water, ethanol, isopropanol, chloroform, butanol and methanol (10% solutions each).

10% (w/v) solvent extracts were prepared by soaking the material over night (1g of root power in 10ml of the respective solvent). The solution was then mixed well using a glass rod and subjected to centrifugation at 5000rmp for 15 min. The supenation collected and used subsequently.

***In vitro* Antimicrobial Assays :** The pour plate culture technique was employed in which the bacteria grow over and within the agar medium. For bacterial culture 4% agar medium was used and for fungal culture different media comprising of 4% potato dextrose was used. The media and the necessary material were autoclaved before use. Various bacterial strains like *E. coli, Staphylococcus, Salmonella* and *Klebisella* obtianed from Veterinary College SKUAST, were used to study antibacterial activity. While as to study the antifungal activity the fungal strains comprised of *Pencillium notatum, Rhizomucor mieche* and *Alternaria alternate,* were obtained from Department of Botany, Kashmir University, Srinagar.

Lipid per oxidation induced by Fe^{2+}/asb : Lipid per oxidation was measured as described by Halliwell *et al.*, 1990. The per oxidation was induced by 5mM $FeSO_4$ and 500mM ascorbate. The products of lipid per oxidation was detected by thiobarbituric acid method measuring the absorbance at 535nm. Incubation was done in the presence of different concentration of extracts.

To 1ml of supernatant obtained above (PMS or microsome) different concentrations of sample material were added. Then 0.2ml of ferric sulphate and 0.2ml of ascorbic acid were added to make total volume 2ml with buffer. The reaction was then stopped by adding 1ml of TCA (20%) followed by addition of 1ml of 1.67% TBA. The mixture was then heated at 100°C for 10 to 20 min. The colour of malondialdehyde-TBA complex in the supernatant was read at 532 nm. The percentage inhibition was calculated by using the followed equation.

$$\% \text{ age inhibition} = \{ (A_c - A_s) / A_C \} \times 100$$

DPPH radical scavenging activity : The assay was carried out according to Sreejayan and Rao (1996). To different concentrations of a sample in methanol, 1ml of a methanol solution of 0.2 mM DPPH was added. After mixing thoroughly, the mixture was allowed to stand in dark for 30 min. and the absorbance at 532 nm was measured using methanol for the baseline correction. The results were then compared with that of the control prepared as above but without added sample. Radical scavenging activity was expressed as a percentage and was calculated by using the following formulae:

$$\% \text{ scavenging} = \{(A_c - A_s)/A_c\} \times 100$$

Super oxide radical scavenging assay : In this assay, the reaction mixture contains 0.07 M phosphate buffer, 0.01M EDTA, 1.72mM NBT and varying concentration of curde extract. The tubes were incubated at 37°C for 5 to 10 min. After incubation 0.12 mM riboflavin was added to each tube and the tubes were then placed in the light for 12-20 min. or 40 W fluorescent light for 7 min. A control tube in which the test sample was replaced by methanol or solvent and the sample blank in which no riboflavin was added was also run in parallel. The absorbance at 560 nm was then measured. The results were expressed as percentage scavenging of the super oxide radical using the following formulae.

$$\% \text{ age scavenging} = \{A_c - [A_s - A_{SB}] / A_C\} \times 100$$

RESULTS

All the extracts with the concentration of 10% (methanol, ethanol, isopropanol, chloroform, aqueous) of root extract of *Saussurea costus* showed antibacterial and antifungal activity which was visible as the zones of inhibition formed in the mixed cultures of Gram positive and Gram negative bacteria in which the extracts were poured down in wells. The results are given in Table (1).

The extracts were selected for further study on anti-bacterial activity against known pathogenic strains of bacteria like *Staphylococcus aureus, E. coli, Salmonella typhimurium* and *Klebsiella penumoniae* as shown inTable (2).

The methanolic and ethanolic extracts of *Saussurea costus* were further subjected to anti-fungal activity against some strains of fungi like *Rhizomucor micehe, Pencillium notatum* and *Alternaria alternata* and the results are shown in Table (3).

From Table (3), it is clear that the 10% methanol and

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ethanol extracts of root *Saussurea costus* show a good antifungal activity against the pathogenic fungi used as text organisms. The results were compared with standard antifungal disc as control (Graseufulvin), which clearly indicates that the root extract of *Saussurea costus* have higher antifungal activity than the antibiotic discs (control) and blank.

DPPH scavenging activity in crude root extract from *Saussurea costus* : The extract and the positive control (Ascorbic acid) showed a significant DPPH free radical scavenging activity as is obvious from the bar diagram shown in Fig.1.

Effect of the oxidant and the plant extract on the lipid per oxidation

Effect of oxidants on lipid peroxidation of PMS

The effect of various oxidants on the lipid peroxidation of the PMS in the presence of the oxidants like Fe^{3+}/A. A along with and without the peroxide H_2O_2, were done in *in vitro* and the effects and shown in the bar diagram shown in the Fig 2 & Fig 3 respectively. It is obvious from the fig that there is the significant effect of the oxidant along with peroxide on the PMS than in the absence of the peroxide.

Effect of root extract from *Saussurea costus* on riboflavin free radical generation : The reaction mixture consisting of EDTA (1.5M), NBT (1.5M), Phosphate buffer (5mM, pH 8), and riboflavin (0.02mM) except control test tube contained extract and showed low absorbance value at 560 nm. The decrease in absorbance was more obvious in case of positive controls having additions of ascorbic acid.

DISCUSSION

Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug (Didry *et al.,* 1998). The presence of antibacterial substances in the higher plants is well established (Srinivasan, 2001).

In order to identify antibiotic properties of *Saussurea costus* against various bacterial and fungal strains, using the disk-agar method, it was established that the plant possesses the potential to inhibit the growth of various pathogens. The inhibition zones produced by the extracts indicated that the plant shows effective antimicrobial and antifungal activities as has been also observed by other (Koul, 1997 and Singh *et al.,* 1983 and Pandey *et al.,* 2005), although the methanolic extract showed higher activity, based on inhibition zone sizes.

There is a strong need for effective antioxidants from natural sources as alternatives to synthetic food additives in order to prevent deterioration of foods, drugs and cosmetics. In this study, the antioxidant properties of the methanol extracts of *Saussurea costus* were examined and were found to be in concordance with the findings of Rawat and Singh (2007).

CONCLUSION

It could be concluded from this study that many plants possess antibacterial activity. We believe that the present investigation together with previous studies provide support to the antibacterial properties of *Saussurea costus.* It can be antibacterial supplement in the developing countries towards the development of new therapeutic agents. The methanolic extract possessed maximum antibacterial and antifungal activities.

The methanol extract of the tested plant *Saussurea costus* was shown to possess radical scavenging activity. However, it was observed that *Saussurea costus* extract caused a dose dependent protection against lipid peroxidation with approximately 70% protection at 1 mg/mL concentration.

There was an approximately 85.2% decrease of the DPPH radical at a 1 mg/ml extract concentration and it was found to be dose dependent.

There was a moderate inhibition of the superoxide radical, with the maximum inhibition being 66% at 1mg/ml extract concentration.

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Table 1 : Antibacterial activity of 10% (w/v) root powder extract of *Saussurea costus* against mixed bacterial culture.

Test Organisms	Methanol	Ethanol	Isopropanol	Chloroform	Aqueous
Mixed population of Gram positive and Gram negative bacteria.	Positive	Positive	Positive	Positive	Positive

Table 2 : Anti bacterial activity of 10% solvent extracts of root of *Saussurea costus* against the test bacteria used.

S. No.	Test organism	Extract used (Root)	Vol. of extract used (µl)	Diameter of zone of inhibition (mm)	Diameter of zone of Inhibition (mm)	
				Extract (test sample)	Antibiotic Disc Control	Blank
1	*E. coli*	Methanol	25	17	16	0
		Ethanol	25	15	16	0
		Isopropanol	25	13.5	17	0
2	*Staphylococcus aureus*	Methanol	25	18	18	0
		Ethanol	25	15	19	0
		Isopropanol	25	15	18	0
3	*Klebisella pnemonae*	Methanol	25	15	17	0
		Ethanol	25	11	17	0
		Isopropanol	25	10	19	0
4	*Salmonella typhimurium*	Methanol	25	14	20	0
		Ethanol	25	13	20	0
		Isopropanol	25	13	20	0

Table 3 : Diameter of zone of inhibition of root extracts of *Saussurea costus* against the test fungal strains.

S. No.	Test organism	Extract used (Root)	Vol. of Extract used	Diameter of zone of inhibition of Extract (mm)	Diameter of zone of Inhibition (mm)	
					Antifungal disc	Blank
1	*Rhizomucor meiche*	Methanol	25	16	0	0
		Ethanol	25	14	0	0
2.	*Pencillium notatum*	Methanol	25	17	0	0
		Ethanol	25	15	0	0
3.	*Alterinaria alternata*	Methanol	25	18	8	0
		Ethanol	25	16	8	0

Table 4 : Effect of methanolic extract of *Saussurea costus* on prevention of oxidation in various *in vitro* assays of antioxidant activities.

Extract concentration (µg / mL)	PMS assay Inhibition (%)	DPPH assay Inhibition (%)	Super oxide Inhibition (%)
Control			
1000	70.7 ± 1.38	85.2 ± 4.0	65.2 ± 4.2
800	61.7 ± 2.45	77.8 ± 4.2	58.4 ± 2.4
600	50.2 ± 2.0	66.5 ± 5.8	50.1 ± 1.5
400	36.2 ± 3.2	45.1 ± 1.2	43.0 ± 1.6
200	30.0 ± 4.2	33.7 ± 1.9	31.3 ± 3.2
Positive control	97.5 ± 4.3	94.3 ± 3.9	84.3 ± 4.2

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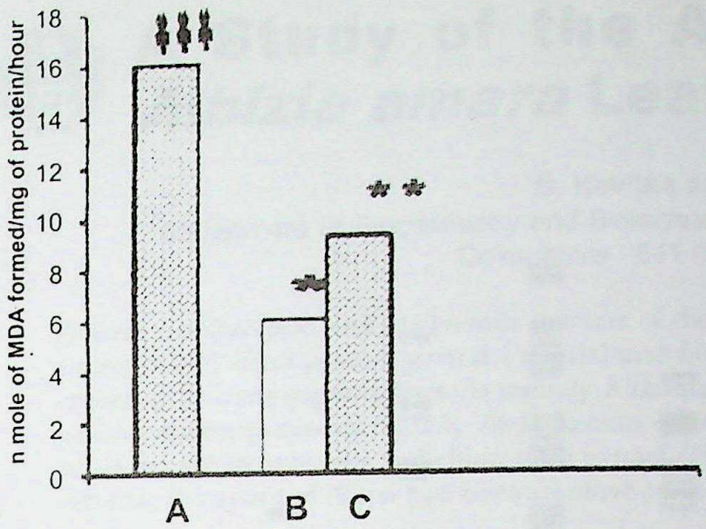

Fig. 1: Effect of *Saussurea costus* (root) methanol and ethanol extracts on DPPH free radical scavenging activity.

A. represents DPPH alone (control), B. represents DPPH and ethanolic extract (root) 250µg, C. represents DPPH and methanolic extract (root) 250µg,
Each value represents mean ± S.E.
*** P < 0.001 with respect to their control.
** P < 0.01 with respect to their control.
P < 0.05 with respect to their control.

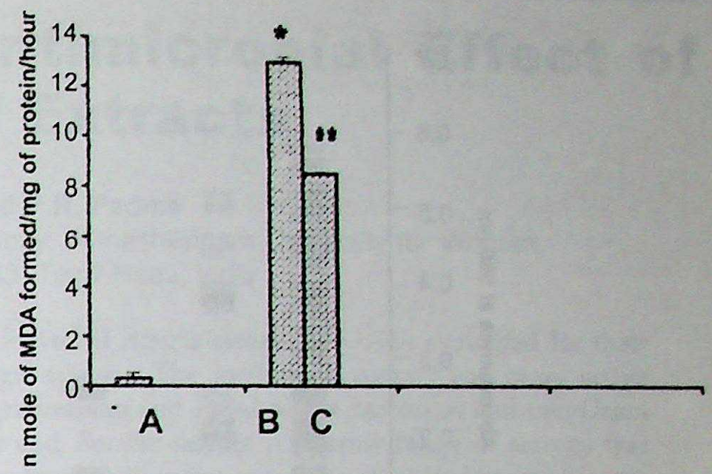

Fig. 2: Post mitochondrial supernatant per oxidations induced by ferric nitrate-ascorbic acid and hydrogen peroxide.

A. represents PMS alone (control), B. represents PMS + Ferric nitrate (20mM) Ascorbic acid (500mM) and Hydrogen peroxide (30mM), C. represents PMS + Ferric nitrate / Ascorbic acid.
Each value represents mean ± S.E
*P < 0.001 with respect to their control.
**P < 0.05 with respect to their control.
P < 0.01 with respect to their control.

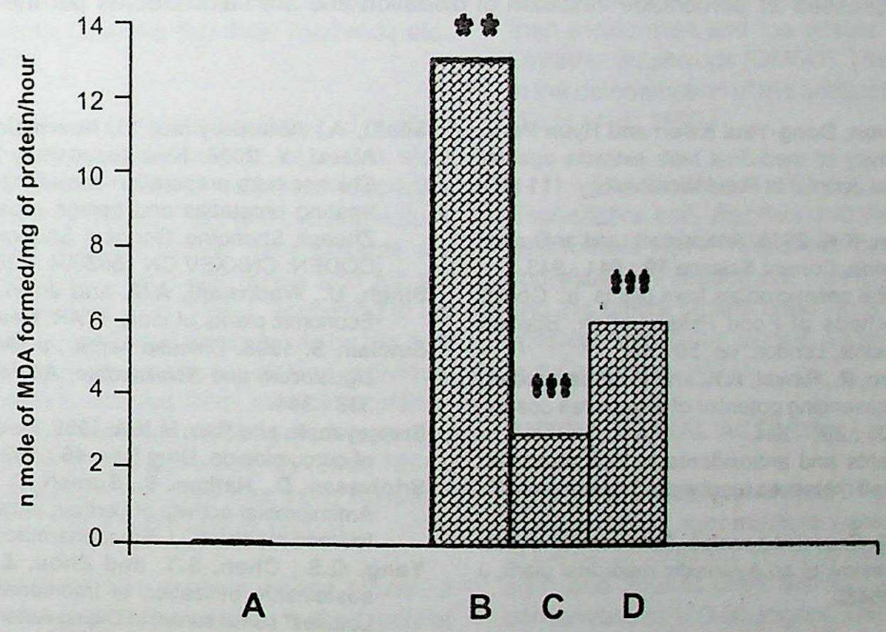

Fig.3. Effect of *Saussurea costus* (root) methanol and ethanol extracts on PMS lipid peroxidation induced by ferric nitrate-ascorbic acid and hydrogen peroxide.

A. represents PMS alone (control), B. represents PMS + Ferric nitrate (20mM) Ascorbic acid (500mM) and Hydrogen peroxide (30mM), C. represents PMS + Methanolic extract (stigma) 250 µg/ml and Ferric nitrate/Ascorbic acid, D. represents PMS ± Ethanolic extract (stigma) 250µg/ml and Ferric nitrate/ Ascorbic acid.
Each value represents mean ± S.E
P < 0.001 with respect to their control.
**P < 0.01 with respect to their control.
***P < 0.05 with respect to their control.

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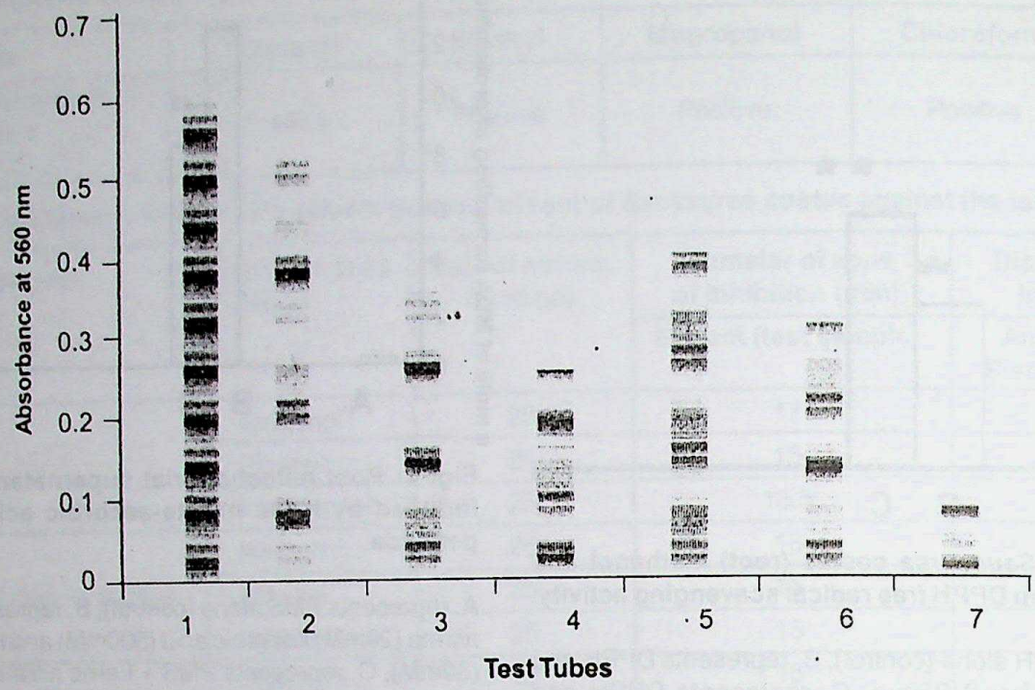

Fig.4: Effect of root extract from *Saussurea costus* on superoxide generation by riboflavin free radical.
Low absorbance value indicated strong antioxidant activity and was proportional to the concentration of extract present. In Fig. test tube 1 represents control, test tube 2,3, and 4 as test solutions having concentration of extract as 250µg/ml, 500µg/ml, 1mg/ml, respectively. While the test tubes 5,6. and 7 represent positive controls having concentration of ascorbic acid as 250µg/ml, 500µg/ml, 1 mg/ml, respectively.

The results are expressed as percentage inhibition of oxidation and are calculated as per the respective formulae as written above.

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A Study of the Antimicrobial Effect of *Albizia amara* Leaf Extracts

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Aqueous, chloroform and methanolic extracts of the leaves of *Albizia amara* have been evaluated for their antimicrobial effect against several bacterial and fungal species. The methanolic extract was more active against two-Gram negative bacteria namely *Klebsiella pneumoniae* and *Pseudomonas fluorescence* and two-Gram positive bacterial species namely *Staphylococcus aureus* and *Bacillus subtilis*. A narrow range of activity was exhibited against aqueous and chloroform extract. The fungistatic activity was also exhibited by methanolic extracts. *Rhizopus* and *Mucor* had been sensitive to the methanolic extracts.

INTRODUCTION

Multidrug resistance towards the antibiotics and their related effects has an added effect to pursue the use of natural drugs. Given the evidence for rapid global spread of resistance of clinical isolates. There is a need for discovery of new antimicrobial agents (Bradford, 2001). Herbal medicine remains the oldest and most used form of medicine of our planet. A rich heritage of knowledge on preventive and curative medicines is available even in ancient scholastic works, like in the Atharvaveda, Rigveda, Charaka Samhita, Ayurveda etc., (Poonkothai *et al.*, 2005).

Plants are the basic source of knowledge of modern medicine. Almost all the parts of the plant, *viz*, bark, root, stem and seeds are known to have various medicinal properties (Chakraborthy and Brantmer, 1999). The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobials (Das *et al.*, 1999). According to the World Health Organisation, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individual medicine have compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998). Earlier literature on antimicrobial activity has been reviewed by Kaushik and Dhiman (2000) and Kaushik (2003).

The plant selected for this study is *Albizia amara*, a moderate sized deciduous tree. It is classified in the family of Leguminosae. It has dark green scaly bark and resembles the acacias but lack thorns. Leaves are abruptly bipinnate; main rachis 5-10cm long densely pubescent.

The plant has been found to possess several ethnomedicinal uses. Dried leaves are powdered and used against dandruff on the scalp. The leaves are also regarded as useful in ophthalmia. The seeds and flowers are also found to have medicinal value for treating several diseases (Sivarajan and Balachandran, 1994).

MATERIAL AND METHODS

Preparation of the extract : The plant specimens were identified, certified and the voucher specimen number (M1) were deposited at the Botanical Survey of India, Southern Circle, Coimbatore. The leaves were shade dried at room temperature and powdered. The powdered leaves were divided into three equal parts of 10g each. Each part was separately subjected to extraction in Soxhlet apparatus for six hours with water, chloroform and methanol respectively. The solvent was then evaporated and the pastes obtained were dissolved in Dimethyl Sulphoxide (DMSO). The solutions were used further in the determination of the antibacterial and antifungal analysis (Silva *et al.*, 1997).

The bacterial strains used for this study were *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas fluorescens, Pseudomonas aeruginosa* and *Shigella flexneri* and the fungal strains used were *Rhizopus stolonifer, Mucor* species, *Aspergillus terreus, Aspergillus fumigatus, Penicillium* and *Candida albicans*.

ANTIBACTERIAL ACTIVITY

Agar Well Diffusion Method : Petriplates containing 20ml of Mueller Hinton agar medium were seeded with a 24hr culture of the bacterial strain. Wells (6 mm diameter) were cut into the agar and 25µl of plant extracts were tested in a series of concentrations 100-500mg/ml. The inoculum size was adjusted so as to deliver a final inoculum of approximately 10^8 colony forming units/ml and incubated at 37°C for 24 hrs. The activity was assessed based on measurement of diameter of inhibition zone formed around the well (NCCLS, 1993).

Determination of Minimum Inhibitory Concentration (MIC): Minimum Inhibitory Concentration was determined by micro dilution method using serially diluted (2-fold) plant extracts according to the National Committee for Clinical Laboratory

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Standards (NCCLS, 2000). The extract was diluted to get series of concentration from 100 mg/ml to 12.5 mg/ml in sterile nutrient broth. One drop of suspension of microorganisms was added to the broth dilutions. These were incubated for 18 hours at 37°C. MIC of each extract was taken as the lowest concentration that did not give any visible bacterial growth. All experiments were performed in triplicate.

ANTIFUNGAL ACTIVITY

Determination of Antifungal Activity : Antifungal activity of *Albizia amara* was demonstrated in a radial growth inhibition assay adapted from the method of Schlumbaum *et al.*, (1986). A fungal plug was placed in the centre of antifungal assay agar plates. Sterile discs were dipped in the extracts and placed over the plates. Nystatin was kept as the positive control. The petriplates were incubated in dark at 23°C. Antifungal activity was observed as a crescent shaped zone of inhibition at the mycelial form. The effect on fungal growth was expressed qualitatively as either strong inhibition (+++) or detectable inhibition (+) or no inhibition (–).

Spore Germination Assay : All the three extracts were tested for the spore germination of different fungi (Rana *et al.*, 1997). Aliquots of spore were prepared by mixing a loopful of spore in 1ml of sterile distilled water. Spores were obtained from a 10 day old culture of fungi. 25µl of spores were added to 10µl extract and placed on separate glass slides. Control was kept in solvents alone. Slides were then incubated in a moist chamber at 25°C for 24 hours. Each slide was fixed in Lacto phenol Cotton Blue stain and observed under the microscope for spore germination.

RESULTS AND DISCUSSION

ANTIBACTERIAL ACTIVITY

The results obtained are summarized in Table 1 indicating the growth inhibition produced by leaf extracts of *Albizia amara* towards 8 species of bacteria of which 6 Gram-negative and 2 Gram- positive. The methanolic extract was found to be more active against two species, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Moderate activity of methanolic extract was found against *Bacillus subtilis* and *Pseudomonas fluorescence*. Aqueous extract showed a little activity *Klebsiella pneumoniae* and *Staphylococcus aureus*. The chloroform extract showed moderate activity against *Bacillus subtilis* and *Pseudomonas fluorescence*.

The highest zone of inhibition was found against *Klebsiella pneumoniae* with methanolic extract and least inhibition against *Pseudomonas fluorescence*.

The results obtained from the study, points out that the active component present in the methanolic extract can prove to be a great remedy for treating diseases. Many ailments like pneumonia, urinary tract infections, meningitis etc. caused by *Klebsiella pneumoniae* and *Staphylococcus aureus* can be treated with the methanolic extract.

The results obtained for Minimum Inhibitory concentration are given in Table 2. This shows that a minimum concentration of 12.5 mg/ml of methanolic extract could resist the growth of *Staphylococcus aureus*. A minimum concentration of 25 mg/ml was required to resist growth of *Klebsiella pneumoniae*. The aqueous extract could inhibit the growth of *Klebsiella pneumoniae* only at a concentration of 50mg/ml. This further validates the activity of 3 extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae*.

ANTIFUNGAL ACTIVITY

Determination of Antifungal Activity : The growth inhibition was determined after 24 hrs and 48 hrs of incubation. The results obtained are summarized in Table 3. Methanolic extract was found to inhibit the growth of all the test organisms after 24 hours of incubation. Growth inhibition of 50% and 25% was recorded for the chloroform and aqueous extracts respectively. Profound inhibition of growth was observed for 2 species *Rhizopus* and *Mucor*. Methanolic extract was found to be active on both fungal species.

Table 1 : Antibacterial activity of aqueous, chloroform and methanolic extracts of leaves of *Albizia amara* by Agar Well Diffusion method.

Microorganisms	Diameter of Zone of Inhibition in mm [Mean ± S.D.]			
	AE	CE	ME	CL
Klebsiella pneumoniae	MS [9.33 ± 0.58]	R	S [13.33 ± 1.15]	29
Staphylococcus aureus	MS [8.0 ± 1.0]	R	S [12.33 ± 0.58]	23
Escherichia coli	R	R	R	22
Bacillus subtilis	R	S [10.67 ± 0.58]	S [10.67 ± 0.52]	27
Pseudomonas fluorescence	R	S [8.33 ± 0.58]	MS [9.33 ± 1.37]	21
Pseudomonas aeruginosa	R	R	R	23
Salmonella typhi	R	R	R	22
Shigella flexineri	R	R	R	21

S → Sensitive, R → Resistant, MS → Moderately Sensitive

AE → Aqueous extract, CE → Chloroform extract, ME → Methanolic extract.

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Table 2 : Minimum Inhibitory Concentration of Aqueous, chloroform and Methanolic Extracts of *Albizia amara* for the two test organisms assayed.

Microorganisms	Minimum Inhibitory Concentration in mg/ml		
	AE	CE	ME
Klebsiella pneumoniae	50	25	25
Staphylococcus aureus	25	25	12.5

ANTIFUNGAL ACTIVITY BY SPORE GERMINATION ASSAY

The results for the spore germination assay are summarized in Table 4. There was significant inhibition of fungal spore germination by methanolic extracts of the leaves of *Albizia amara*. Aqueous extract showed inhibition against two species only, that is *Rhizopus* and *Aspergillus fumigatus*. Only a single fungal species, *Mucor* responded to the activity of chloroform extract.

Table 3 : Antifungal activity of Aqueous, Chloroform and Methanolic extracts of leaves of *Albizia amara*.

Microorganisms	Growth Inhibition							
	Aqueous		Chloroform		Methanol		Nystatin	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
Rhizopus stolonifer	++	+	–	–	+++	++	+++	+++
Mucor sp.	–	–	++	+	+++	++	+++	+++
Aspergillus fumigatus	++	+	++	+	++	+	+++	+++
Aspergillus terreus	–	–	–	–	+	–	+++	+++
Penicillium	–	–	++	+	+	–	+++	+++
Candida albicans	–	–	–	–	++	+	+++	+++

+ → <50% inhibition, +++ → 100% inhibition, ++ → ≥50% inhibition, – → No inhibition

Table 4 : Antifungal activity of Aqueous, Chloroform and Methanolic Extracts of leaves of *Albizia amara* by Spore Germination Assay.

Microorganisms	EXTRACTS			
	Aqueous	Chloroform	Methanol	Control
Rhizopus stolonifer	+	–	+	+
Mucor sp.	–	+	+	+
Aspergillus fumigatus	+	–	+	+
Aspergillus terreus	–	–	+	+
Penicillium	–	–	+	+

+ → Germination, Control [solvent alone] – → No Germination

This assay also validates the potential of methanolic extract of *Albizia amara* leaves against pathogenic fungal species.

All the antimicrobial studies conducted above clearly showed the significance of the methanolic extract. The active chemical present in the methanolic extract can be an ideal candidate for treating diseases caused by pathogenic fungal species. Diseases like mucormycosis caused by *Mucor* and *Rhizopus* can be alleviated by the methanolic extract of the leaves of *Albizia amara*.

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Antimicrobial Activity of Medicinal Plants Against Vaginal Pathogens

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The various solvent extracts of the bark of *Ficus religiosa, Ficus benghalensis, Ficus gloomerata,* the fruits of *Tribulus terrestris* and the roots of *Saussurea lappa* were investigated for their antimicrobial activity against the isolated and ATCC strain of *Staphylococcus aureus, Escherichia coli* and *Candida albicans.* The antimicrobial activity was determined in Petroleum ether, Chloroform, Methanol and Aqueous extracts of plants. Amongst all the extracts tested, methanolic extract of all the five plants was comparably more effective to inhibit the growth of microbes than Petroleum ether, Chloroform and Aqueous extracts. The extracts were subjected for antimicrobial activity against the vaginal pathogens at 200mg/ml concentration by disc diffusion method. Of all the three microorganisms *E. coli* and *S. aureus* showed good antimicrobial activity. Phytochemical analysis of the bark, fruits and roots extracts revealed the presence of many biologically active constituents such as alkaloids, flavonoids, glycosides, steroids, tannins, phenols and saponins. These support a good response to the use of these plants in herbal medicine and as a base for the development of new drugs and phytomedicine. The antibacterial activities of extracts were compared with standard antibiotics. Of all the plants tested *Tribulus terrestris* was found to show the best antimicrobial activity than the other.

Keywords : Antimicrobial activity, Disc diffusion method, Phytochemicals.

INTRODUCTION

Biologically active compound from natural source has always been of great interest to scientists working on infectious diseases. In recent years there has been a growing interest to evaluate plants possessing antimicrobial activity. Plant derived drugs serves as a prototype to develop more effective and less toxic medicines. Infection with various microorganisms is one of the leading causes of a number of diseases. Vaginitis is an inflammation of the vagina that can result in discharge, itching and pain. The cause of vaginitis depends on the type of vaginal infection a women have *i.e.*, bacterial infection and yeast infection. Certain causative microorganisms of bacterial infection are *Trichomonas vaginalis, Chlamydia, Gardnerella vaginalis, Streptococcus pyogenes, Staphylococcus aureus* and sexually transmitted organisms, these either alone or in combination are responsible for vaginal discharge. Yeast infection occurs when certain internal or external factors change the normal environment of the vagina and trigger an overgrowth of a microscopic fungus - the most common being *Candida albicans.*

India has a treasure of medicinal plants and a number of herbs are traditionally used for the treatment of many diseases. Thus in recent years there has been a phenomenal rise in the interest of scientific community to explore the pharmacological activities of medical plants (Chah *et al.*, 2006). *Ficus* genus consists of over 800 species and belongs to family Moraceae. *Ficus religiosa* (Pipal). *Ficus benghalensis* (Bargad) and *Ficus gloomerata* (Gular) are large deciduous trees distributed throughout India and their parts like bark, leaves, tender shoots, fruits, seeds and latex are medicinally important. The bark is used in inflammations, swelling of neck, gonorrhoea, scabies mouth wash for tooth ache for strengthening gums. *Tribulus terrestris* (Gokhru) is a flowering herb belongs to family Zygophyllaceae, native to warm temperature and tropical regions of the world of Asia, Africa, Europe, Australia and America. Its fruits are used in folk medicine as tonic, analgesic, astringent, stomachic, anti-hypertensive, diuretic and urinary anti-infective (Ody, 2000). *Saussurea lappa* (Kustha) belongs to family Asteraceae, grow abundantly on the Himalayas and valley of Kashmir. The root has a pungent taste and a peculiar fragrant aromatic odour. It has been used in cough, asthma, chronic rheumatism and skin diseases, fever, dyspepsia and cholera.

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals and plants. One of such resources is herbal medicines. The present paper deals with the antimicrobial activity and phytochemical analysis of *Ficus religiosa, Ficus benghalensis, Ficus gloomerata. Tribulus terrestris* and *Saussurea lappa* extracts.

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MATERIAL AND METHODS

The bark of the *Ficus* plants were collected from the plants growing wild in Hardwar (U.K.), in September 2007, the fruits of *Tribulus terrestris* were collected from the plants growing in the botanical garden of Patanjali Yog Peeth, Hardwar in September 2007 and the roots of *Saussurea lappa* were collected from Shivalik range of Himalayas in October 2007 and identified at Botanical Survey of India, Dehradun, Uttarakhand. Fresh plant materials were washed under running tap water, shade dried at room temperature and then pulverized to get a coarse powder and used for successive extraction. The plant extracts were prepared by immersing 200gm of dried powder in 600ml of solvents *i.e.*, Petroleum ether, Chloroform, Methanol and Water by Soxlet apparatus. At the end of extraction each extract was passed through Whatmann Filter Paper No. 1. The extracts were concentrated by using vacuum evaporator at 30°C and stored in sterile bottles at 4°C until further use.

The pathogenic organisms were selected for the study on the basis of their clinical pharmaceutical importance as well as for their potential to cause infection and contamination of food and durgs. *S. aureus*, *E.coli* and *C.albicans* were isolated from infected females with vaginal infections in Subharati Institute of Medical Sciences, Meerut, *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *C.albicans* ATCC 10231 were purchased from National Chemical Lab (NCL), Pune. The isolates were identified according to the published guidelines by Burnette *et al.*, 1994.

The antimicrobial activity was evaluated by Agar Disc Diffusion Method given by Bauer *et al.*, in 1966. The disc of 6mm was saturated with 45µl (200mg/ml) of the plant extract, solvent blank and antimicrobial drug, ciprofloxacin for bacteria and clotrimazole for fungi, allowed to dry and was introduced on the upper layer of the seeded agar plate. All the solvents served as negative control. The plates were then incubated for 18-24 hours at 37°C and microbial growth was determined by measuring the diameter of zone of inhibition in millimeters (mm).

Phytochemical Screening : The phytochemical analysis of plant extracts were carried out by using standard qualitative methods given by Trease and Evans, 1996 and Scalbert, 1991.

RESULTS AND DISCUSSION

The presence of antibacterial substances in the higher plants is well established (Srinivasan *et al.*, 2001), Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. The present study has shown that medicinal plants possess antimicrobial properties that support their value in herbal medicine for the treatment of diseases and ailments. Table 2 shows the effect of the different extracts of the plants against the tested pathogens at a concentration of 200 mg/ml. All the plant extracts showed significant antimicrobial activity against all the microorganisms.

In *Ficus religiosa*, methanol extract showed the maximum, antimicrobial activity against the *E. coli* (16mm) followed by chloroform, aqueous and petroleum ether extract and minimum against ATCC strain of *C.albicans* (11mm). In *Ficus benghalensis* maximum antimicrobial activity was shown by methanol extract against *S.aureus* (16mm) and minimum against isolated and ATCC strain of *C.albicans* (12mm) followed by aqueous, chloroform and petroleum ether extracts. In *Ficus gloomerata* the methanol extract showed good activity against the isolated culture of *E. coli* (16mm) and least against ATCC strain of *C. albicans* (11mm) followed by aqueous, chloroform and petroleum ether extract.

In *Tribulus terrestris* all the extracts showed the significant activity against all the tested microorganisms. The extract.obtained in methanol showed maximum antimicrobial activity against *S. aureus* (17mm) followed by aqueous, chloroform and petroleum ether. In *Saussurea lappa* the petroleum ether extract did not show any activity against the isolated and ATCC strain of *S. aureus*. Maximum activity was shown in methanol extract against the isolated culture of *E.coli* (15mm) and least was shown in isolated and ATCC strain of *S. aureus* (12mm).

Table 1 shows the phytochemical profile of different extracts of *Ficus religiosa, Ficus benghalensis, Ficus gloomerata, Tribulus terrestris* and *Saussurea lappa* which contain alkaloids, flavonoids, glycosides, steroids, tannins, phenols and saponins. The antimicrobial activity can be due to the presence of these phytochemicals. Plant compounds with antimicrobial properties can be used as antimicrobial agents in new drugs for the therapy of infectious diseases.

All the plants showed greater inhibitory activity in the methanolic extracts when compared to petroleum ether, chloroform and aqueous extracts. The most susceptible strains were *E.coli* and *S.aureus* isolated from vaginal infection. Alcohol was found to be a better solvent for extraction of active substances in comparison to other solvent. Chloroform extract generally shows lower activity than methanolic or aqueous extracts and they inhibited a smaller range of bacteria (Pesewu *et al.*, 2008). In the present work the antimicrobial activity of the extracts was quantitatively assessed by the presence or absence of the zone of inhibition and the plants selected are potentially a rich source of antimicrobial agents. Successive isolation of chemical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in this study the plant extracts by methanol provided more consistent antimicrobial activity.

Depending on the nature of the infecting microorganism, the condition may vary from minor ailments to major health concern. The present study has shown that the part of the medicinal plants possess antimicrobial properties that support their value in herbal medicine. The results indicated differential activities of the plant extracts against the growth of bacteria and fungi. In conclusion our results would support the efficacy of these for the treatment of vaginal infection.

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Table 1 : Phytochemical screening of various extracts of *Ficus* species, *Tribulus terrestris* and *Saussurea lappa*.

Name of constituents	*Ficus religiosa*				*Ficus benghalensis*				*Ficus gloomerata*				*Tribulus terrestris*				*Saussurea lappa*			
	P	C	M	W	P	C	M	W	P	C	M	W	P	C	M	W	P	C	M	W
Alkaloids	+	+	+	+	-	-	-	-	+	+	-	+	-	-	+	+	+	-	+	+
Flavonoids	-	-	+	-	+	+	+	+	-	-	+	+	+	-	+	-	-	-	+	-
Glycosides	-	+	+	-	-	-	+	+	+	+	-	-	+	+	+	+	-	+	+	-
Saponins	-	+	+	+	-	-	+	+	-	-	+	+	-	+	+	+	-	-	-	+
Steroids	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+
Phenols	+	-	+	+	-	-	+	+	+	+	+	-	+	+	-	+	+	-	+	+
Tannins	-	-	+	-	+	-	+	-	-	+	-	+	-	+	+	+	-	+	+	+

P - Petroleum ether, C - Chloroform, M - Methanol, W - Water
+ = Present , – = Absent

Table 2 : Antimicrobial activity of *Ficus religiosa, Ficus benghalensis, Ficus gloomerata, Tribulus terrestris and Saussurea lappa* extracts (mm).

S. No.	Name of Pathogens	*Ficus religiosa*				*Ficus benghalensis*				*Ficus gloomerata*				*Tribulus terrestris*				*Saussurea lappa*				Ciprofloxacin* Clotrimazole**
		P	C	M	W	P	C	M	W	P	C	M	W	P	C	M	W	P	C	M	W	
1	*S. aureus**	13	12	14	12	13	12	16	14	14	14	14	13	13	14	17	15	--	12	12	13	20
2.	*S. aureus* ATCC*	11	13	13	12	15	14	15	15	13	13	13	14	12	15	15	13	--	11	12	14	21
3.	*E. coli**	13	15	16	12	13	13	14	13	13	13	16	14	13	12	14	13	13	13	15	14	24
4.	*E. coli* ATCC*	11	14	16	13	14	13	14	15	13	14	15	12	14	13	14	10	11	12	13	15	23
5.	*C. albicans***	11	11	12	13	11	09	12	12	12	11	12	11	10	11	13	09	12	11	13	12	22
6.	*C. albicans* ATCC	10	10	11	14	10	11	10	10	10	13	11	11	09	13	12	10	12	10	13	14	22

Average of three replicaties

P - Petroleum ether, C - Chloroform, M - Methanol, W - Water

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Isolation of Plant Growth Promoting Rhizobacteria and Fungi Resistant to Heavy Metal Contaminants

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In this study biosorption of Iron, Zinc, Lead, Copper and Magnesium ions by PGPR isolates from sugar mill effluent contaminated soil were investigated. PGPR were identified and characterized as *Bacillus* spp, *Pseudomonas* spp, *Azotobacter* spp, *Glucanoacetobacter* spp, *Phosphobacteria* spp, and fungi, *Aspergillus niger* and *Penicillium* spp. All the isolates exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) for heavy metals ranging from 50µg/ml to 350µg/ml. The effects of pH and temperature on the biosorption capacity of microorganism were investigated. The optimum pH and temperature values for biosorption of heavy metals for bacteria was observed as pH 6,7 and temperature 37°C, for fungi the optimum biosorption capacity was identified as pH 5 and temperature 28° C respectively. The maximum adsorption was achieved on Iron at initial concentration 100 mg Iron (Fe^{2+}/l), followed by Zinc (100 mgZn^{2+}/l). Lead (100mg Pb^{2+}/l), Copper (100mg Cu^{2+}/l) and Magnesium (100mg Mg^{2+}/l) and under these conditions the biosorption values indicated that *Bacillus* spp, *Pseudomonas* spp, *Aspergillus niger* and *Penicillium* spp were suitable for biosorption of heavy metals from effluents.

Keywords : Biosorption, Heavy metals, Effluent, PGPR.

INTRODUCTION

The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Copper, Chromium, Nickel, Lead, Zinc and Cadmium are known to be the most commonly used heavy metals and the most widespread contaminants of the environment. Metals are directly or indirectly involved in all aspects of microbial growth, metabolism and differentiation. Many metal such as K, Na, Mg, Ca, Mn, Fe, Cu, Ni, Co, Zn, Mo are essential for biological functions, where as it is not known whether some others, such as Al, Ag, Cd, Sn, Au, Sr, Hg and Pb have essential biological functions. All these elements can interact with microbial cells and be accumulated as a result of physiochemical mechanisms and transport systems of varying specificity, independent on or directly and indirectly dependent on metabolism (Gadd, 1988). Some of these processes are of biotechnological importance being relevant to metal removal and recovery from mineral deposits and industrial effluents for industrial use or environmental bioremediation.

Metals are introduced into aquatic systems as a result of the weathering of soils and rocks, from volcanic eruptions, and from a variety of human activities involving in the mining, processing, or use of metals or substance that contain metal contaminants (Laws, 1993). The last group which has the highest potential of toxicity and pollution includes waste waters coming from metal plating industry, automobile, electrical and electronic materials, home appliance, pipes, caps, guns, mechanics, dye, sugar mill and tannery industries. Unlike other pollutants, heavy metals are getting important because of the fact that they can not be decomposed by *in situ* biological means. Virtually all metals, including the essential metal micronutrients, are toxic to aquatic organisms as well as humans if exposure levels are sufficiently high (Laws, 1993), It is, therefore, necessary to remove heavy metals from waste waters before discharge due to effects of disturbing environmental quality and being harmful to human health.

The removal of metals from industrial effluents can be achieved by ion exchange, chemical oxidation, chemical precipitation etc (Aksu *et al.*, 1992). Different physico-chemical methods such as active carbon adsorption, ion exchange and reverse osmosis are used. As an alternative to these methods, recently the method of the removal of heavy metal contaminants by means of bacteria has been focused on. Biological romoval of heavy metal contaminants from aquatic effluents offers great potential when metals are present in trace amounts (Vinita and Radhanath, 1992).

The bioremediation of heavy metals using microorganisms has received a great deal of attention in recent years, not only as a scientific novelty but also for its potential application industry. Many microbial species such as bacteria, fungi, yeast and algae are known to be capable of adsorbing heavy metals on their surface or accumulating within their structure (Camphell and Martin 1990). Physical adsorption or

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ion exchange at the living or non-living cell surface is very rapid and occur into contact with heavy metal ions. Accumulation occurs in living cells and is slow, related to metabolic activity (Gadd, 1990). Among the microorgansim used PGPR play an important role in bioremediation. Plant growth promoting rhizobacteria (PGPR) are a heterogeneous. group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly or indirectly. In last few decades a large array of bacteria including species of *Pseudomonas, Azospirillium, Azotobacter, Klebsiella, Bacillus, Enterobacter* and *Serratia* have been reported to enhance plant growth (Kolepper *et al.*, 1989). Although there are a number of studies on removal of heavy metals, the knowledge of the applications of biosorbents in the environments and industry has not yet been clear yet.

MATERIAL AND METHODS

(a) Sample Collection : The sampling area was the soil contaminated by sugar mill effluent of Cuddalore district. Samples was collected in sterile plastic bottles and brought to the lab for study.

(b) Isolation and identification of heavy metal resistant PGPR isolate : For the selective isolation of heavy metals resistant microorganism, heavy metal incorporated media were used. The resistant isolate were identified as *Bacillus* spp, *Pseudomonas* spp, *Azotobacter* spp, *Glucanoacetobacter* spp, *Phosphobacteria* spp, and fungal species, *Aspergillus niger* and *Penicillium* spp. The isolates were grown on selective isolation agar incorporated with heavy metals like Fe^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+} and Mg^{2+} prepared. The concentration of each heavy metal was maintained at 50µg/ml of the medium. After the incubation period the plates were observed. The isolated and distinct colonies were sub cultured and obtained in the form of pure culture.

(c) Determination of Minimum Inhibitory Concentration (MIC) : MIC of PGPR isolate grown on heavy metals incorporated media was determined by gradually increasing the concentration of the heavy metal by 10µg/ml each time in the specific media until the strains failed to give colonies on the plate. The starting concentration used was 50µg/ml. The culture growing on the last concentration was transferred to the higher concentration by streaking on the plate. MIC was noted when the isolates failed to grow on plates.

(d) Growth of Microorganisms and Biosorption : The bacterial and fungal isolate was incubated at 37°C and 28°C at 150 rpm for 40-48 hours in the selective broth. At the end of incubation biomass was separated from medium by centrifuging at 5000 rpm and it was kept in the oven at 50°C to remove the free water as much as possible. Then, it was suspended in deionized water in order to use it in the biosorption. 100ml solutions containing 100mg/l (Cu^{2+}, Fe^{2+}, Zn^{2+}, Pb^{2+} and Mg^{2+}) were prepared individually from stock solution containing 1g/l Cu^{2+} ($CuSO_4$), 1g/l Fe^{2+} ($FeSO_4$), 1 g/l Zn^{2+} ($ZnSO_4$) respectively. Then 4ml respective cultures were added to the medium and adsorptions of metals were investigated for different pH and temperature, values were adjusted by using HCl and NaOH at 37°C and 28°C. The solution containing the biomass was agitated in a shaker of 150rpm during the adsorption. The analyses of heavy metals were carried out by atomic adsorption spectrophotometer (Perkin-Elmer) at 0.01 ppm sensitively level after dilution of the samples.

By taking the determined optimum conditions into consideration, the copacity of microorganism to remove the mentioned metal from industrial waste water was searched with the same method.

RESULTS AND DISCUSSION

Isolation and identification of Heavy metal resistant bacteria : Heavy metal resistant PGPR *(Bacillus* spp, *Pseudomonas* spp, *Azotobacter* spp, *Glucanoacetobacter* spp, *Phosphobacteria* spp, *Aspergillus niger, Penicillium* spp*)* were isolated from sugar mill effluent contaminated soil (Cuddalore district) against heavy metal like Iron, Zinc, Lead, Copper and Magnesium. All the isolates exhibited high resistance to heavy metals ranging from 50µg/ml to 350µg/ml. All the isolates showed multiple tolerances to heavy metal. Minimal Inhibitory concentration test indicated highest to copper (300µg/ml) by *Pseudomonas* spp, (180µg/ml) by *Bacillus* spp, (150µg/ml) by *Azotobacter* spp, (110µg/ml) by *Glucanoacetobacter* spp, (80µg/ml) for *Phosphobacteria* spp for fungi the highest inhibitory concentration were seen on copper (150µg/ml) by *Aspergillus* niger and (130µg/ml) by *Penicillium* spp.

Biosorption : For biosorption of Iron, Zinc, Lead, Magnesium and Copper the optimum condition (pH and temperature) determined was pH 6 and 7, temperature 37°C for bacteria and for fungi pH 5 and 28°C. All the isolate exhibited high biosorption activity on heavy metal Iron, Zinc, Lead, Magnesium and Copper. Obtained result are shown in Table (1 - 9).

Under the optimum condition determined (Table 1-8) the highest uptake, % 100 yield, was determined for Iron ions. Biosorption of the metals in their individual solutions by *Bacillus* spp, *Pseudomonas* spp, *Azotobacter* spp, *Glucanoacetobacter* spp, *Phosphobacteria* spp, *Aspergillus niger* and *Penicillium* spp was determined to be Iron, Zinc, Lead followed by Magnesium and Copper. The walls of becteria and fungi are efficient metal chelators (Gadd, 1990).

The study was focused on biosorption of Iron ions due to high recovery rate of Iron ions by PGPR isolate. Because Iron ions were fully biosorbed from solution having a 100mg/l initial concentration, experiments were carried out on the lead solution of 250 mg/l as initial concentration in order to apply the process of waste waters (Semra Hhan *et al.*, 2004). The soil collected from sugar mill (Cuddalore district) was used as sample. The Minimal inhibitory concentration for the removal of Iron, Zinc, Lead, Magnesium and Copper ion from the effluent by PGPR were studied and given in Table 9, while in the individual solutions the biosorption yield of the metals by PGPR was (110, 140, 150, 180, 180µg/ml) by *Bacillus* spp followed by (200, 250, 260, 280, 300µg/ml) by *Pseudomonas* spp, (120,

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Table 1 : Effect of pH 3 on Biosorption of Heavy metals.

Isolates	Iron (100mg/l)	Zinc (100mg/l)	Lead (100mg/l)	Magnesium (100mg/l)	Copper (100mg/l)
Bacillus spp	90.1	90.6	92.4	93.1	93.6
Pseudomonas spp	86.4	87.0	89.0	89.2	90.6
Azotobacter spp	94.5	95.0	97.6	97.6	97.9
Glucanoacetobacter spp	96.0	96.0	97.0	97.4	97.9
Phosphobacteria spp	97.2	98.2	98.5	99.0	99.0
Aspergillus niger	87.5	88.4	89.0	89.2	91.5
Penicillium spp	89.7	89.5	90.0	91.4	92.7

Table 2 : Effect of pH 4 on Biosorption of Heavy metals.

Isolates	Iron (100mg/l)	Zinc (100mg/l)	Lead (100mg/l)	Magnesium (100mg/l)	Copper (100mg/l)
Bacillus spp	89.4	89.9	90.1	91.1	92.5
Pseudomonas spp	85.6	86.6	89.6	89.2	90.1
Azotobacter spp	92.1	93.5	91.4	96.6	97.1
Glucanoacetobacter spp	94.3	95.7	97.1	97.4	98.6
Phosphobacteria spp	93.2	95.2	95.5	96.0	97.0
Aspergillus niger	84.3	85.3	86.4	87.2	88.1
Penicillium spp	86.9	87.5	86.9	89.6	90.6

Table 3 : Effect of pH 5 on Biosorption of Heavy metals.

Isolates	Iron (100mg/l)	Zinc (100mg/l)	Lead (100mg/l)	Magnesium (100mg/l)	Copper (100mg/l)
Bacillus spp	84.9	85.2	85.4	86.2	87.2
Pseudomonas spp	81.2	82.3	84.2	86.2	87.2
Azotobacter spp	92.4	93.5	95.4	97.6	97.6
Glucanoacetobacter spp	94.3	94.7	96.2	97.4	97.6
Phosphobacteria spp	92.2	94.2	95.2	95.0	97.0
Aspergillus niger	82.3	83.3	84.4	85.4	87.1
Penicillium spp	86.0	87.5	87.9	88.6	89.5

Table 4 : Effect of pH 6 on Biosorption of Heavy metals.

Isolates	Iron (100mg/l)	Zinc (100mg/l)	Lead (100mg/l)	Magnesium (100mg/l)	Copper (100mg/l)
Bacillus spp	72.1	75.6	76.69	77.4	79.19
Pseudomonas spp	67.8	69.8	69.9	70.0	70.5
Azotobacter spp	83.2	85.7	89.2	89.6	90.8
Glucanoacetobacter spp	86.1	88.9	89.9	90.3	94.8
Phosphobacteria spp	88.6	89.9	90.4	91.2	94.8
Aspergillus niger	66.3	68.5	69.2	69.8	70.2
Penicillium spp	69.2	70.3	71.7	72.5	73.6

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Table 5 : Effect of pH 7 on Biosorption of Heavy metals.

Isolates	Iron (100mg/l)	Zinc (100mg/l)	Lead (100mg/l)	Magnesium (100mg/l)	Copper (100mg/l)
Bacillus spp	69.5	71.9	72.0	73.1	74.6
Pseudomonas spp	66.2	68.8	69.9	70.4	71.3
Azotobacter spp	80.5	83.4	85.2	86.6	87.8
Glucanoacetobacter spp	86.7	88.5	89.1	90.3	91.3
Phosphobacteria spp	88.4	89.5	91.2	91.4	92.8
Aspergillus niger	64.3	66.7	68.2	68.8	69.5
Penicillium spp	67.5	67.1	69.1	69.4	71.6

Table 6 : Effect of Temperature (28°C) on Biosorption of Heavy metals.

Isolates	Iron (100mg/l)	Zinc (100mg/l)	Lead (100mg/l)	Magnesium (100mg/l)	Copper (100mg/l)
Bacillus spp	92.5	93.3	94.2	95.3	96.5
Pseudomonas spp	90.4	92.1	93.8	94.2	95.5
Azotobacter spp	96.9	98.8	98.9	99.2	99.5
Glucanoacetobacter spp	97.1	98.3	100.0	100.0	100.0
Phosphobacteria spp	98.2	98.6	100.0	100.0	100.0
Aspergillus niger	76.3	78.2	79.6	80.2	81.9
Penicillium spp	78.7	81.0	82.6	83.4	85.5

Table 7 : Effect of Temperature (32°C) on Biosorption of Heavy metals.

Isolates	Iron (100mg/l)	Zinc (100mg/l)	Lead (100mg/l)	Magnesium (100mg/l)	Copper (100mg/l)
Bacillus spp	96.9	97.0	98.1	98.2	98.4
Pseudomonas spp	95.0	96.8	97.6	97.8	97.9
Azotobacter spp	98.7	98.9	99.0	100.0	100.0
Glucanoacetobacter spp	99.8	99.7	100.0	100.0	100.0
Phosphobacteria spp	99.8	100.0	100.0	100.0	100.0
Aspergillus niger	94.1	95.8	96.0	96.5	97.4
Penicillium spp	93.7	94.6	95.5	95.8	97.8

Table 8 : Effect of Temperature (37°C) on Biosorption of Heavy metals.

Isolates	Iron (100mg/l)	Zinc (100mg/l)	Lead (100mg/l)	Magnesium (100mg/l)	Copper (100mg/l)
Bacillus spp	72.1	73.6	74.0	75.5	77.8
Pseudomonas spp	70.1	71.8	72.6	73.5	74.6
Azotobacter spp	79.4	80.6	82.3	83.0	85.4
Glucanoacetobacter spp	83.5	85.5	85.9	86.7	87.1
Phosphobacteria spp	84.2	85.2	85.6	86.0	86.9
Aspergillus niger	87.7	89.7	90.1	90.5	91.3
Penicillium spp	88.2	90.2	91.2	91.2	92.5

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Table 9 : Minimum inhibitry concentration of isolate to heavy metals.

Isolates	Resistance	MIC (µg/ml)
Bacillus spp	Iron	110
	Zinc	140
	Lead	150
	Magnesium	180
	Copper	180
Pseudomonas spp	Iron	200
	Zinc	250
	Lead	260
	Magnesium	280
	Copper	300
Azotobacter spp	Iron	120
	Zinc	130
	Lead	140
	Magnesium	140
	Copper	150
Glucanoacetobacter spp	Iron	60
	Zinc	80
	Lead	90
	Magnesium	90
	Copper	110
Phosphobacteria spp	Iron	60
	Zinc	60
	Lead	70
	Magnesium	80
	Copper	80
Aspergillus niger	Iron	60
	Zinc	80
	Lead	90
	Magnesium	120
	Copper	150
Penicillium spp	Iron	70
	Zinc	90
	Lead	100
	Magnesium	120
	Copper	130

130, 140, 140, 150µg/ml) by *Azotobacter* spp, (60, 80, 90, 90, 110 µg/ml) by *Glucanoacetobacter* spp, (60, 60, 70, 80, 80µg/ml) by *Phosphobacteria* spp, (60, 80, 90, 120, 150µg/ml) by *Aspergillus niger* and (70, 90, 100, 120 and 130µg/ml) by *Penicillium* spp respectively. Because the adsorption was selective (Nakajima and Akaguchi 1989) and high in the industrial waste waters having low ion concentration, it can be said that PGPR isolate can be used in tertiary treatment of Sugar mill effluent contaning heavy metals at low concentration (Vinita and Radhanath, 1992).

As a result, it can be concluded that PGPR isolate can be used in the removal or recovery of heavy metal ions, especially Iron ions from effluent. It can also be said that the usage of PGPR can be successful to remove Zinc, Lead, Magnesium and Copper element from the effluent containing high level of heavy metals. Further studies needed to increase the biosorption capacities of biomass and to develop appropriate technologies applicable in the treatment of effluent.

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Antimicrobial Efficiency of *Costus igneus* N.E.Br. (Costaceae)

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Costus igneus were subjected to soxhlet extraction using methanol, chloroform and petroleum ether solvents. The extracts were tested for the presence of various phytoconstituents and antibacterial activity by Agar well diffusion method. All the extracts have shown antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and Gram-nagative (*Escherichia coli*). The minimum inhibitory concentration was determined for each of the extract against the tested bacteria. It was found that the methanol extract was high inhibiting the tested bacteria followed by chloroform and petroleum ether extracts in most cases. Antifungal activity is carried out for *A. niger* and *C. keratinophilum* both of them showed moderate activity. The antimicrobial activity of solvent extracts of selected plants may be attributed to the presence of various phytochemicals present in them.

Keywords : Antibacterial activity, Methanol, Phytochemical, Bacteria, Fungi and *Costus*.

INTRODUCTION

Plants used in traditional medicine may constitute an important source of new biologically activity compounds. Utilization of plants for medicinal purposes in India has been documented in ancient literature (Ravindra and Vijay Kumar, 2007). The use of remedies from plant origin covers a wide veriety of maladies and constitutes an alternative way to antibiotics, Which is otherwise no longer promising due to the generalized antibiotic resistance among pathogenic microorganisms. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the microorganisms. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Phytomedicines derived from plants have shown great promise in the treatment of various diseases which includes viral infections (Bhagath *et al.*, 2008). *Costus igneus* N.E. Br. Common name fiery costus or spiral slag is an herbaceous belongs to Costaceae family. It is a succulent perennial herb, growing up to 2.7 m high and having an erect stem. The plant possesses horizontal rhizomatous rootstock. It generally grows luxuriantly on clay loam soil near inland forest under moderate shade. The plant propagates vegetatively through rhizomes or via seeds dispersed by birds. Rhizome is the plant part used as medicine. In ayurveda or via seeds dispersed by birds. Rhizome is the plant part acrid, cooling, aphrodisiac, purgative, antihelminthic, depurative, febrifuge, expectorant and tonic (Edward, 1999). The present work evaluating the phytochemical constituents and antimicrobial properties of *Costus igneus*.

MATERIAL AND METHODS

Collection and Extraction of plant material : *Costus igneus* N.E. Br. were collected from Lakkavali region of Chikmaglure district. Karnataka.The plant were authenticated in Department of Applied Botany. Jnanasahyadri, Shankaraghatta and voucher specimen (KU/AB/KSV/45) deposited in the Department for future reference. The plant materials were washed thoroughly 2-3 times with running tap water and once with sterile water, shade dried, powdered and used for extraction. The powdered plant material was extracted with solvents namely petroleum ether, chloroform and methanol. A known amount of powdered material (500gm) was subjected to soxhlet extraction and exhaustively extracted with respective solvents for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the desiccator (Kumaraswamy *et al.*, 2008 and Manjunatha *et al.*, 2006). The extracts were subjected to preliminary phytochemical screening to screen the presence of various secondary metabolites in the solvent extracts (Kokate, 2004, Roberts *et al.*, 1981 and Parekh & Chanda, 2007).

Preliminary screening of solvent extracts for antibacterial activity : Gram-positive (*Staphylococcus aureus* MTCC-902 and Gram-negative (*Escherichia coli* MTCC-405 and *Pseudomonas aeruginosa* MTCC-1934) bacteria's were used in the antibacterial study. The bacteria were procured from Institute of Microbial Technology, Chandigarh. The bacteria were screened for their sensitivity towards the plant extracts by Agar well diffusion method (Tepe *et al.*, 2004). In this method,

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24 hours old Muller-Hinton broth cultures of test bacteria were swabbed unifomly on solidified sterile Muller-Hinton agar plates using sterile cotton swab. Aseptically wells of 6 mm dia were done in the inoculated plates with the help of gel puncher. Different solvent extracts (1mg/ml to DMSO), Standard (Chloramphenicol and Tetracycline 1mg/ml) and Control (DMSO) were added into the respectively labeled wells. The plates were incubated at 37°C for 24 hours in upright position, after incubation zone of inhibition was recorded. The experiment was carried out in triplicated to get average reading.

Determination of Minimum inhibitory concentration (MIC) : The MIC of the solvent extracts against test bacteria was tested by Agar well diffusion method (Tepe *et al.*, 2004). The 24 hours old standardized Muller-Hinton broth cultures of test bacteria were swabbed uniformly on solidified sterile Muller-Hinton agar plates using sterile cotton swab. Wells of 6 mm dia were done in the inoculated plates with the help of gel puncher. Various dilutions of the plant extracts (10, 25, and 50µg/ml of DMSO) were added into the respectively labeled wells. The plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition was recorded. The least dilution among four was considered as the MIC. The experiment was carried out in triplications to get average reading.

Antifungal assay : Fungi namely *Aspergillus niger* (MTCC478), *Chrysosporium keratinophilum* (MTCC1367) were tested for their susceptibility to the solvent extracts. All these cultures were obtained from MTCC collection IMTECH (Institute of Microbial Technology), Chandigarh, India. The cultures were maintained at 4°C and subcultures frequently in respective media. The antifungal activity of plant extracts by Agar well diffusion method (Tepe *et al.*, 2004). In this method, spore suspension of test fungi, in 0.01% Tween 80 in saline, were swabbed uniformly on solidified sterile Sabouraud's dextrose agar plates using sterile cotton swab. Aseptically wells of 6 mm diameter were bored in the inoculated plates with the help of sterile gel puncher and the extract (10mg/ml of DMSO), Standard (fluconazole, 1mg/ml) and Control (DMSO) were added into the respectively labeled wells. The plates were incubated at 28°C for 72 hours in upright position and the zone of inhibition was recorded. The experiment was carried in triplicates to get average reading.

RESULTS AND DISCUSSION

The presence of various secondary metabolites in the methanol extract of plants selected for study is given in Table-1. The phytoconstituents namely Tannins, steroids and glycosides were found in the *Costus igneus* but flavonoids, alkaloids and saponins were absent.

The results of antimicrobial acitvity of solvent extracts were shown in Table – 2. Results show the presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of microbial growth and it was reported as positive and absence of zone as negative. It was found that all the solvent extracts of the plants *C. igneus* were found to inhibit growth of test microorganisms. Standard antibiotic Chloramphenicol (1mg/ml) for Gram+ve bacteria and Tetracycline (1mg/ml) for Gram - ve has shown inhibition of all tested bacteria and Flucanazole for Fungi. The control solvent DMSO did not revel inhibition of any of the tested bacteria.

Preliminary screening showed positive results for the antimicrobial activity, with the help of preliminary screening, minimal inhibitory concentrations (MIC) of extracts wree done. MIC of different solvents extracts was shown in Table-3. From the table it is clear that the methanol extracts of the plants are more effective in inhibiting test bacteria then others. The methanol extract of concentration 25 µg/ml was effective in inhibiting 50% of test bacteria. Following methanol extract, petroleum ether extract showed inhibition of test bacteria at low concentration when compared to chloroform extract in most cases. Among Gram-negative bacteria, *P.aeruginosa* has shown susceptibility at higher concentration (50µg/ml) then others.

MIC of different solvent extracts was shown in Table-4. Methanol extracts were found to possess more fungal activity followed by petroleum ether and chloroform extracts. Among tested fungi *A. niger* was showed more activity in methanol extract followed by *C. keratinophilum*. Chloroform extract of *C. igneus* was not found effective against *C. keratinophilum*. Previously reported that the rhizome of *Costus speciouse* as a constitutive principle with antifungal activity against *Cladosporium cucumerinum, Colletotricum, Curvularia* sp. and *Penicillium* sp. (Bandara *et al.*, 1988). It has been clearly exhibited in our study.

The antimycotic activity of extracts could be due to the presence of various phytoconstituents. Interest in plants with antimicrobial properties has reviewed as a result of current problems associated with the use of antibiotics. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005). In our study, the extracts have shown promising results in terms of inhibition of bacteria tested. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infections diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant (Fabricant and Farnsworth, 2001).

Table 1 : Phytochemical constituents in the methanol extracts of selected plants.

Phytochemical group	*C. igneus*
Tannins	+
Terpenoid	–
Alkaloid	–
Steroid	+
Saponins	–
Flavonoids	–
Glycosides	+

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Table 2 : Preliminary screening for Antimicrobial activity of solvent extracts of *C. Igneus*.

Microorganisms	Pet. Ether Extract	Chloroforn Extract	Methanol Extract	Control	Standard
E. coli	+	+	+	–	+
S. aureus	+	+	+	–	+
P. aerugenosa	+	+	+	–	+
A. niger	+	+	+		+
C. keratinophilum	+	+	+		+

'+' Inhibition, '–' No inhibition

Table 3 : Minimum inhibitory concentration (MIC) of solvent extracts of *C. igneus*.

Bactera	Pet. Ether extract			Chloroform extract			Methanol extract			Control	Standard
Conc. (µg/ml)	50	25	10	50	25	10	50	25	10		
E.coli	3	1	1	2	1	–	4	2	2	–	8
S. aureus	2	2	–	2	–	–	3	2	1	–	6
P. aerugenosa	3	2	2	2	2	–	3	1	–	–	6

Note: Results are average of three trials, Minimum inhibitory concentration

Table 4 : Minimum inhibitory concentration (MIC) of solvent extracts of *C. igneus*.

Fungi	Pet. Ether extract	Chloroform extract	Methanol extract	Control	Standard
A. niger	3 ± 0.4	4 ± 0.3	7 ± 0.3	–	10
C. keratinophilum	2 ± 0.4	0.3 ± 0.2	4 ± 0.2	–	7

Note : Results are average of three trials.

CONCLUSION

From the study it is found that the plants used in the study possess marked antifungal and antibacterial activity in terms of inhibition of fungal and bacterial growth *in vitro*. The results of the study have justified the traditional use of the plants in curing diseases. A further study on animal models has to be carried out.

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Antimicrobial and Antiplasmid Activities of Clove Extract and Eugenol

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Antimicrobial activity of cloves and eugenol was tested by agar-disc-diffusion method and MIC (minimum inhibitory concentration) by tube dilution method against non-pathogenic and resistant pathogenic gram-positive (*Staphylococcus aureus, Streptococcus pneumoniae, Bacillus cereus*) and gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris*) and fungi *Candida albicans, Aspergillus niger* and *Fusarium oxysporum*. Eugenol was more potential in killing bacteria and fungi than clove extract. The MIC of clove extract ranged between 12.5 - 25µg/ml and eugenol 0.2 - 1.0µg/ml for all the bacteria and fungi tested. Membrane leakage of UV_{260} (nucleic acid) and UV_{280} (proteins) absorbing material from bacteria into extracellular medium gradually increased after treating the bacteria with the test compound and proved that this may be one of the modes of bactericidal action. The time course of lethal action was also studied against *E. coli* and *Staphylococcus aureus*. Clove extract took 240 min and 210 min for effective (100%) killing of *E. coli* and *S. aureus* respectively where as eugenol took only 45min for effective killing of both the bacteria. Studies on elimination of β-lactamase enzyme activity and antiplasmid activity was done using *E. coli* HB101 but neither the clove extract nor the active principle (Eugenol) possess the property of eliminating either β-lactamase activity or drug resistant plasmid.

Keywords : Eugenol, MIC, β-lactamase, antiplasmid, antimicrobial etc.

INTRODUCTION

Cloves (*Syzygium aromaticum*, syn. *Eugenia aromaticum* or *Eugenia caryophillata*) are the aromatic dried flower buds of an ever green tree belong to the family Myrtaceae (Srivastava and Malhotra, 1991). In India, cloves are grown in Nilgiri, Tenkasi - hills and in Kanyakumari district of Tamilnadu state. It is also cultivated in Kottayam and Quilon districts of Kerala. Cloves are used in ayurveda, Chinese medicine and western herbalism. Cloves contain about 15 to 20% of volatile oil/essential oil, 10 to 13% tannins (gallatonic acid), resin, chromone and eugenin. The volatile oil of the clove contains 70 to 90% of eugenol, eugenyl acetate, caryophyllenes and small quantities of esters, ketones and alcohols (Chaieb *et al*, 2007).

Cloves are carminative (Phyllis and James, 2000), antioxidant (Lee and Shibamoto, 2001), antiulcerogenic (Bae *et al*, 1998) and antithrombotic (Srivastava and Malhotra, 1991).The volatile oil of clove eugenol is a strong germicide, a powerful antiseptic, a feeble local anesthetic applied to decayed teeth, and as a dental analgesic (Sashidhar, 2002), also used to treat acne, warts, and scars.

Clove oil is also an effective mosquito repellent (Trongliki *et al*. 2005), fragrant and flavouring agent (Chaieb *et al*, 2007). However, clove oil is toxic to human cells. If ingested or injected in sufficient quantity, may cause life threatening complications, including acute respiratory distress syndrome, hepatic failure and CNS disorders (Kirseh, 1990; Lane *et al* 1991). In the light of above information it was proposed to compare antimicrobial and antiplasmid properties of clove extract and eugenol, as plasmids are often responsible for drug resistance and resistance to many β-lactam antibiotics is often caused by β-lactamase enzyme.

MATERIAL AND METHODS

Plant material : Cloves were purchased from local market and Eugenol from Sigma chemicals ltd.

Bacterial cultures : Non-pathogenic Gram-positive bacteria - *Staphylococcus aureus* (NCIM -2079), *Bacillus cereus* (NCIM -2016) and *Streptococcus pneumoniae* (NCIM -2080).

Non-pathogenic Gram-negative bacteria - *Escherichia coli* (NCIM -2089), *Klebsiella pneumoniae* (NCIM -2957), and *Pseudomonas aeruginosa* (NCIM 2200), *E. coli* HB101 were obtained from NCIM, NCL, Pune.

Resistant pathogenic bacteria - *E. coli* - 1 was isolated from a patient's urine sample and *Staphylococcus aureus* - 1 from pus of an infected person.

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Fungal cultures : *Candida albicans, Aspergillus niger* and *Fusarium oxysporum* were obtained from Department of Botany, Osmania University, Hyderabad.

Chemicals and Media: Purchased from Qualigens and Hi-Media laboratories India ltd.

Extraction of Cloves : The cloves were ground to a fine powder before extraction. The Clove powder (50g) was then extracted with acetone using soxhlet apparatus by continuous heat extraction for 24hrs. The extract obtained was concentrated to dryness by evaporating the solvent under reduced pressure (Shastry *et al*, 2005). The concentrate thus obtained was dissolved in dimethyl sulfoxide (DMSO) such that the final concentration of the extract would be 1g/ml of DMSO.

Antimicrobial screening by disc diffusion method : The *in vitro* antimicrobial activity of acetone extract of clove and eugenol were carried out by disc diffusion method (Elgayyar *et al*, 2001). Actively growing log phase cultures were mixed in soft nutrient agar (Nutrient broth with 1% agar) and fungal spores in potato dextrose agar and plated. The 1µl of clove extract (1mg/1µl) and 1µl of eugenol were loaded onto different filter paper discs prepared from Whatmann No 1 filter paper. The discs were then placed on the agar medium containing the cultures, and incubated for 24hrs at 37°C for bacteria and at 28°C for 2 to 3 days for fungi. The diameter of zone of growth inhibition was recorded. The effects were compared with that of the standard antibacterial ampicillin (10µg/disc) and antifungal clotrimazole (10µg/disc). DMSO alone served as control.

Minimum inhibitory concentration : MIC was determined by serial dilution method (Stokes, 1975). Two fold serial dilution of the test compounds were carried out in the nutrient broth. To each test tube an inoculum containing 10^5 cells/ml of actively growing bacterial cultures in log phase was added. The culture tubes were incubated at 37°C for 24hrs. After the incubation, a loop full of treated cultures of bacteria are streaked on nutrient agar media and incubated for 18hrs at 37°C. The plates were checked for the growth of bacteria and MIC of the test compound was determined and expressed in µg/ml.

Determination of time course of lethal action on bacteria : *E.coli, Staphylococcus aureus* were treated with test compounds at MIC values and at regular time intervals aliquots of treated cultures were drawn, diluted appropriately and plated on nutrient agar plates. The inoculated plates were incubated at 37°C for 24hrs for colony development. The number of colonies were counted and the number of viable cells was determined.

Mode of action of clove extract and eugenol on bacteria : The method of Heipieper (1992) was followed to determine the leakage of UV_{260} and UV_{280} absorbing material. The cell suspension was prepared. *S. aureus* cells were grown overnight with continuous shaking in nutrient broth at 37°C, harvested, washed with 10mm EDTA and then twice in distilled water by centrifugation each time at 6000rpm for 15 minutes at 4°C and resuspended such that the absorbance of the final suspension was 2.0 at A_{450}. After incubation for 30mins at room temperature, the test clove extract and eugenol were added separately to suspension at MIC concentration. At regular intervals of time aliquots of the samples were drawn, centrifuged and UV_{260} and UV_{280} absorbing material in the supernatant was measured.

Test for development of resistance for bacteria against clove extract and Eugenol : Log phase culture of *E.coli* was exposed to ½ MIC of clove extract and eugenol for 18hrs at 37°C and then transferred to fresh medium containing ½ MIC of test compound. In this manner, the culture was passed 3 times and then the culture was streaked on nutrient agar media plates containing 1 MIC of test compound and then incubated for 24hrs at 37°C. If growth occurred it indicated that organism developed resistance and, if no growth occurred it indicated that no resistance was developed against test compounds.

Effect of test compound on β-lactamase enzyme activity: Few bacterial strains develop resistance against β-lactam drugs by producing an enzyme β-lactamase or pencillinase. This enzyme converts biologically active penicillin or ampicillin to an inactive penicilloic acid which adsorbs iodine. The adsorption or non-adsorption of iodine can be detected by starch indicator system. Inhibition of β-lactamase in bacteria (*E. coli* HB101) was studied as per the procedure of Odugbemi *et al.* (1977).

Antiplasmid activity : Test for elimination of plasmid by clove extract and eugenol was carried according to the procedure described by Winkler *et al.* (1979) was done in three steps. (1) Treatment of the drug resistant *E.coli* HB101 containing PBR322 plasmid responsible for Amp^r and Tet^r at ½ MIC of the test compound. (2) Extraction of plasmid from the treated and untreated bacteria cultures. (3) Visual confirmation (presence or loss) of plasmid by agarose-gel electrophoresis using UV transilluminator.

RESULTS AND DISCUSSION

The acetone clove extract and eugenol was studied for their antimicrobial properties *in vitro*. The disc diffusion method revealed that the MIC of the clove extract ranged between 12.5 - 25µg/ml for all the bacteria tested. *S. aureus* was found to be more sensitive (12.5µg/ml) than other Gram-positive bacteria, where as the entire Gram-negative bacteria tested were equally sensitive and the MIC was 25µg/ml. Eugenol is more efficient than clove extract; the MIC is 0.2 to 0.8µg/ml. *S.aureus* was more sensitive followed by *St.pneumoniae* and *B.cereus* (0.2, 0.6 and 0.8µg/ml respectively) among the Gram-negative bacteria, *Klebsiella pneumoniae* and *Proteus vulgaris* are sensitive followed by *E.coli* (0.4 & 0.8µg/ml respectively). The time course of lethal action studied against *E.coli* and *S.aureus* showed that clove extract took 240min & 210min for 100% killing of *E.coli* & *S.aureus* respectively and Eugenol took only 45min for 100% killing of both the bacteria (Fig 1 & 2). Membrane leakage of UV_{260} & UV_{280} absorbing material from *S.aureus* cells into extra cellular medium proved that this

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Table 1 : Antibacterial activity of clove extract and eugenol by disc-diffusion method.

S.No.	Micro organisms	Zone of growth inhibition in mm		
		Clove extract (1mg/µl)	Eugenol (1µl)	Ampicillin (10µg/disc)
1.	*Staphylococcus aureus*	19.0	20.0	22.0
2.	*Streptococcus pneumoniae*	12.3	14.5	13.0
3.	*Bacillus cereus*	11.7	14.0	19.0
4.	*Escherichia coli*	10.0	12.0	18.0
5.	*Klebsiella pneumoniae*	13.0	14.0	20.0
6.	*Pseudomonas aeruginosa*	12.8	18.0	24.0
7.	*Proteus vulgaris*	11.0	12.0	18.5
8.	*Escherichia coli* (urine)	9.0	11.0	8.0
9.	*Staphylococcus aureus* (pus)	16.0	20.0	NI

Table 2 : Antifungal activity of clove extract, eugenol and clotrimazole (for comparison) by disc-diffusion method.

S.No.	Micro organisms	Zone of growth inhibition in mm		
		Clove extract (2.5 mg)	Eugenol (1µl)	Clotrimazole (10µg/disc)
1.	*Candida albicans*	13.5	13	14
2.	*Aspergillus niger*	11.0	20	11
3.	*Fusarium*	12.5	21	10.3

Table 3 : Minimum inhibitory concentration of clove extract, eugenol and ampicillin (for comparison) in µg/ml.

S.No.	Micro organisms	Zone of growth inhibition in mm		
		Clove extract	Eugenol	Ampicillin
1.	*Staphylococcus aureus*	12.5	0.20	0.98
2.	*Streptococcus pneumoniae*	25.0	0.60	1.96
3.	*Bacillus cereus*	25.0	0.80	31.2
4.	*Escherichia coli*	25.0	0.80	1.96
5.	*Klebsiella pneumoniae*	25.0	0.40	0.98
6.	*Pseudomonas aeruginosa*	25.0	0.40	0.98
7.	*Proteus vulgaris*	25.0	0.80	31.2
8.	*Escherichia coli* (urine)	50.0	1.60	125
9.	*Staphylococcus aureus* (pus)	200.0	6.40	>250

may be one of the modes of lethal action. UV_{260} absorbing Nucleic acid material leakage gradually increased after 15min, but UV_{280} absorbing proteins leakage was initially slow but increased after 90min (Fig 3 & 4). Development of resistance by bacteria *E. coli* against both clove extract and Eugenol was interestingly not noticed even after 10 time's serial passage in ½ MIC of the test compounds. Both clove extract and Eugenol could not inhibit β-lactamase enzyme activity and eliminate drug resistant plasmid from the bacteria (Fig 5). The present study are in harmony to those reported by Burst & Reinders (2003) that clove oil was antibacterial (Lopez *et al*, 2005, Sashidar, 2002).

The present study and earlier reports reveals that cloves and the active principle of cloves, eugenol are effective antimicrobials. Findings from current study support the use of cloves in traditional medicine for the treatment of various bacterial and fungal infections and also local herbal or traditional medicinal industries modernizing their production systems.

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Fig.1: Time course of lethal action of clove extract and eugenol against *S.aureus*.

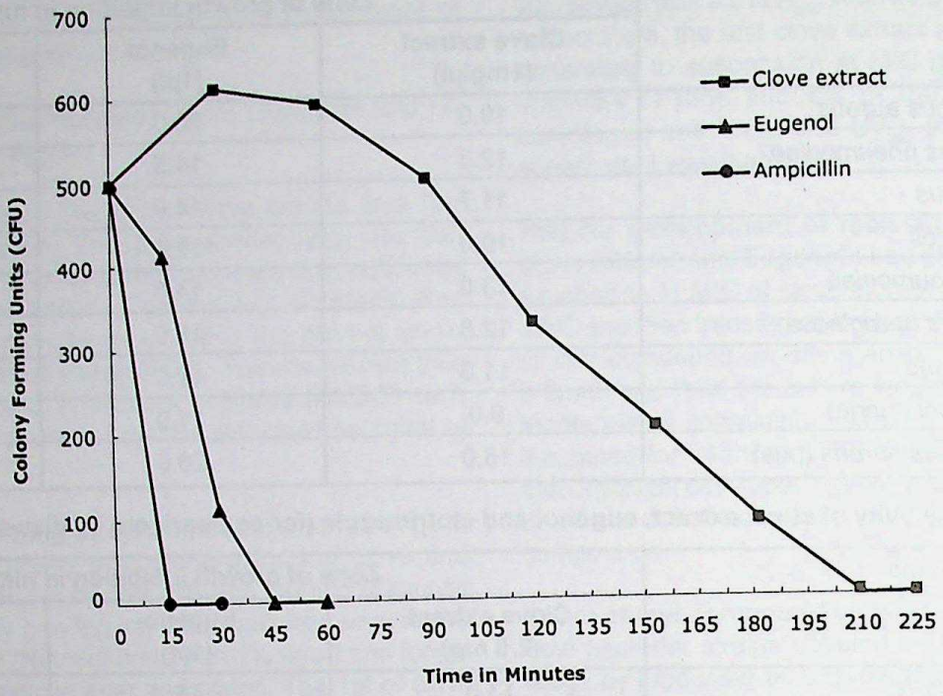

Fig. 2: Time Course of lethal action of clove extract and eugenol against *E.coli*.

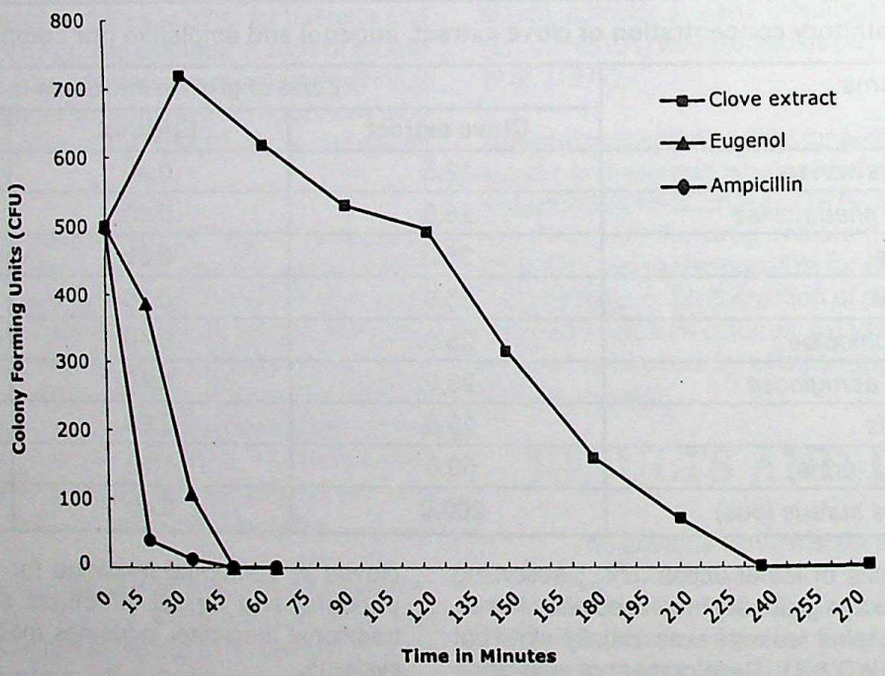

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Fig. 3: Effect of clove extract and eugenol on leakage of UV_{260} nm absorbing material from *S.aureus* cells at MIC.

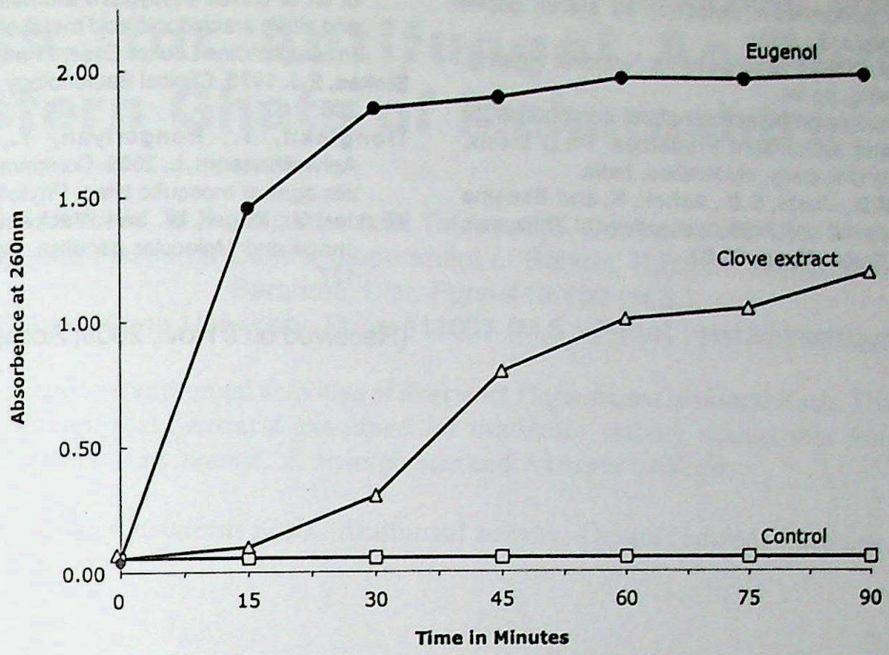

Fig. 4: Effect of clove extract and eugenol on leakage of UV_{280} nm absorbing material from *E. coli* cells at MIC.

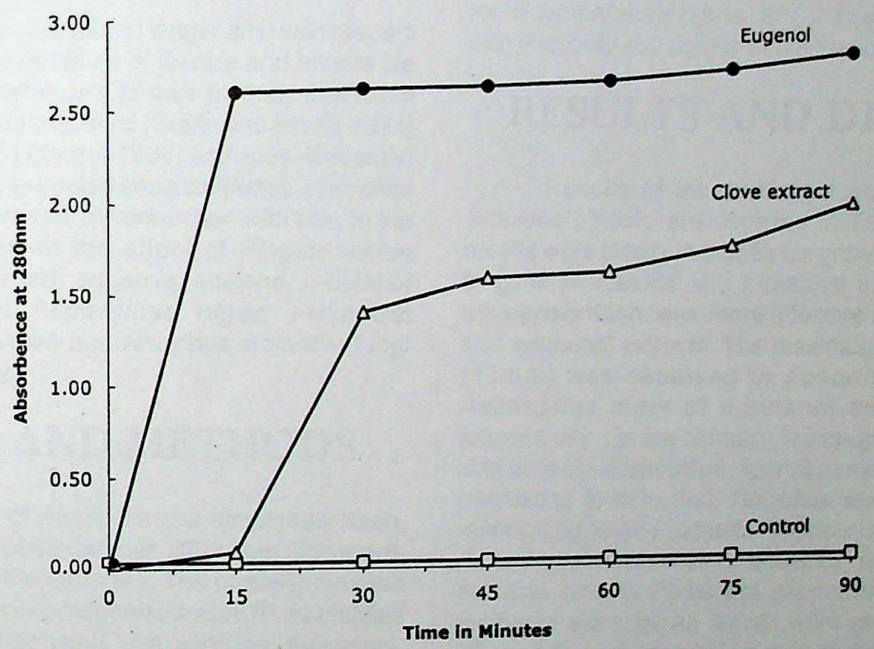

Fig. 5: Agarose gel electrophoresis bands in detection of plasmid after treatment with clove extract and Eugenol.

Lane 1 : Plasmid (1kb λ marker)

Lane 2 : Clove extract

Lane 3 : Eugenol

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Screening of *Plagiochasma simulensis* Kash. for Antifungal Activities from Western Ghats of Maharashtra

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Present work is depends on antifungal activities of liverwort *Plagiochasma simulensis* Kash. The aqueous, acetone, n-butanol and methanol plant extracts examined for inhibitory activity against four fungal pathogens like *Aspergillus niger, Fusarium graminearum, F. moniliformae* and *Rhizopus stolonifer.*

Keywords : *Plagiochasma simulensis* Kash., Antifungal activity, Organic solvent fractions, Western Ghats, Maharashtra.

INTRODUCTION

Bryophytes remain unexplored longer time with respect to antimicrobial properties because of its rare and unique life cycle. Number of bryophytes are known to their medicinal (Chang and Shao, 1986), antibacterial (Gupta and Singh, 1971) and antibiotic (Chopra and Bhatla, 1990) activities. Recently, Asakawa (1994 and 2001) extracted sesquiterpenes, phenolics and terpinoids from bryophytes. In connection with this, in the present study we examined the effect of *Plagiochasma simulensis* Kash. extracts with aqueous, acetone, n-butanol and methanol against *Aspergillus niger, Fusarium graminearum, F. moniliformae* and *Rhizopus stolonifer* fungi to prove antifungal activity.

MATERIAL AND METHODS

The plant material of *Plagiochasma simulensis* Kash. was collected in the month of September, 2009 from Sinhagarh, region of Western Ghats, Maharashtra. The properly cleaned (10 g) of plant material was homogenized with 10 ml distilled water as well as organic solvents like acetone, n-butanol, methanol separately. Homogenized material was shake on rotary shaker (250-300 rpm) for 24 hrs and filtered it with double layered muslin cloth and make 20 ml final volume with respective solutions (pH - 7). Filtrate was stored in refrigerator for further use in antifungal assays.

Antifungal properties of extracts were tested against the aforesaid fungi by filter paper disc diffusion method (Ronald *et al.*, 1984). Sterile Whatmann No. 1 paper discs (6 mm) were saturated with respective extracts then dried and placed on fungi inoculated Sabourd's agar plates. On each agar plate three filter paper discs were kept. Antifungal activity was assessed by measuring the diameter of growth inhibition zone surrounding of the discs up to 72 hrs at an interval of 24 hrs at room temperature (28 to 30°C). The results were compared with Penicillin (50 µg/ml) treated as control.

RESULTS AND DISCUSSION

Results of the antifungal activity of *Plagiochasma simulensis* Kash. are depicted in Table-1 and Plate-1. The results were clearly indicates the growth inhibition of test fungi. In general acetone and n-butanol extract of *Plagiochasma simulensis* Kash. was more effective as compare to aqueous and methanol extracts. The maximum growth inhibition zone (13mm) was observed in *Fusarium graminearum* and *Aspergillus niger* of n-butanol and methanol extracts respectively. On the contrary, minimum growth inhibition zone was noted in *Aspergillus niger* (6 mm) of aqueous extract. It is interesting to note that, *Rhizopus stolonifer* growth was not responding to any extracts. Trace inhibition was recorded in *Fusarium moniliformae* of aqueous, n-butanol and methanol extracts and in *Fusarium graminearum* of acetone and methanol extracts. All results were compared with Penicillin (50 µg/ml) as a control but growth inhibition of all test fungi was altogether absent in it.

Joshi *et al.* (1990) observed the effect of *Exermotheca tuberifera* Kash. extracts on 04 bacterial strains *viz., Escherichia coli, Klebsiella pneumonae, Bacillus substilis* and *Staphylococcus aureus* and reported growth inhibition of all test bacterial strains. The *in-vitro* antifungal activity of *Pallavicinea lyelli* was studied by Subhisha and Subramoniam (2005) against four test fungi (*Aspergillus niger, A. fumigatus, Fusarium oxysporum* and *Candida albicans*) using disc diffusion method. They concluded that water, alcohol and hexane extracts of *Pallavicinea lyelli* showed varying levels of

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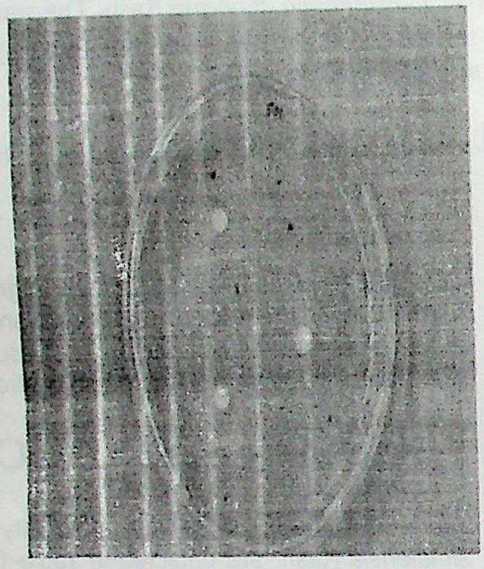
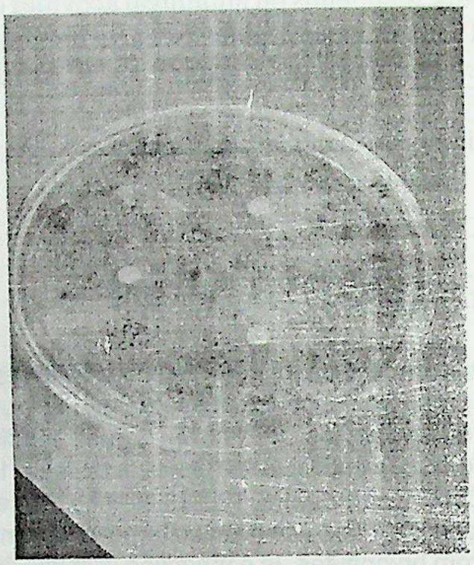
Table 1 : Antifungal screening of *Plagiochasma simulensis* Kash. against test fungi using disc diffusion method.

Nature of *Plagiochasma simulensis* Kash. extract	Diameter of inhibition zone of fungi (mm)			
	Rhizopus stolonifer	*Fusarium moniliformae*	*Fusarium graminearum*	*Aspergillus niger*
Control (Penicillin 50 µg/ml)	–	–	–	–
Aqueous	–	±	–	6
Acetone	–	8	±	9
n-butanol	–	±	13	9
Methanol	–	±	±	13

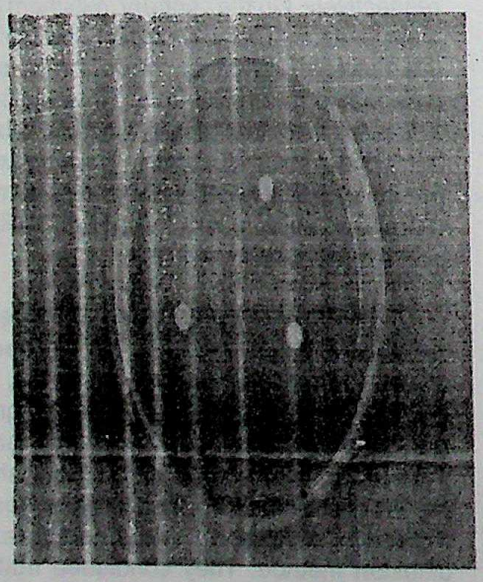
Plate 1 : Antifungal effect of *Plagiochasma simulensis* Kash. extracts against fungal strains.



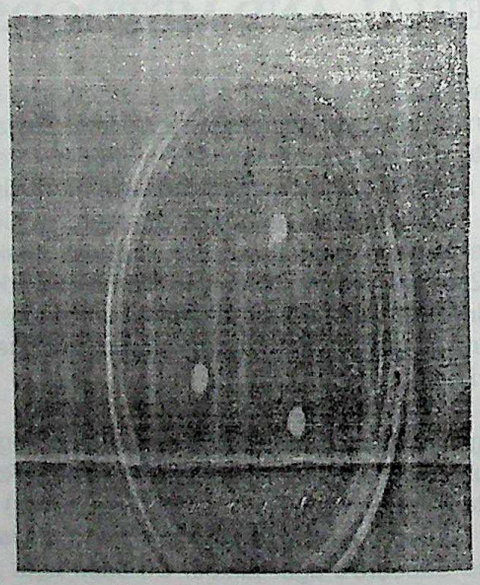
Fusarium moniliformae **(acetone extract)**



Fusarium graminearum **(n-butanol extract)**



Aspergillus niger **(n-butanol extract)**



Aspergillus niger **(methanol extract)**

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activity against the test fungi; the alcohol extract exhibited the maximum activity. Out of four fungi, *Aspergillus fumigatus* was found to be most sensitive.

Different liverworts and mosses screened to determine their antimicrobial activity against the selected bacteria and fungal pathogenic species were studied by Bodade *et al.* (2008) and recorded promising effect on growth inhibition of test organisms. Aqueous, methanol, n-propanol and petroleum ether extracts of 40 Cyanobacterial isolates belonging of nine genera were examined for inhibitory activity against five fungal pathogens (*viz., Aspergillus niger, A. flavus, Colletotrichum musae, Paecilomyces lilacinus* and *Fusarium oxysporum*) by Pawar and Puranik (2008).

The antimicrobial activity of bryophytes depends on the physiological age of the species, season of the collection and ecological conditions in which they are growing (Joshi *et al.*, 1990). Therefore variations are observed in such studies. In the present work we also find out the antifungal activity of *Plagiochasma simulensis* Kash. against four test fungi. It is hoped that this piece of work will unravel many intricate problems pertaining to antibiotic properties of liverworts and will also give a guideline to feature work.

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Evaluation of Suitable Concentration of PGRs on Callus Induction, Proliferation, Somatic Embryogenesis and Regeneration in *Jatropha curcas* L.

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The present study was undertaken to evaluate the most suitable concentration of growth regulators *viz*, 2, 4-D, IBA, BAP, Kinetin, IAA and NAA for callus induction, its proliferation, somatic embryogenesis and regeneration in explants of *Jatropha curcas* L. Good callus formation was obtained on MS medium containing 0.93µM Kinetin and 0.73 µM IBA. Excellent embryogenesis was observed at 2.15µM NAA and 4.44µM BAP from endosperm explants. Rapid *in vitro* propagation by shoot proliferation on internodal segments isolated from field grown mature plants was obtained at 2.32µM Kinetin and 6.66µM IBA.

Keywords : *Jatropha curcas,* Callus induction, Somatic embryogenesis, Regeneration.

INTRODUCTION

J. curcas L. a well known biofuel plant belongs to family Euphorbiaceae, commonly known as Physic nut is a native to America and Africa. *J. curcas* seed contain 37% oil (Heller, 1996). The oil can be combusted as fuel without being refined. It burns with clear smoke-free flame, tested successfully as fuel diesel engine. The by-products are press cake, a good organic fertilizer, oil contains also insecticide. In recent years, the plant is increasingly grown as a substitute for biodiesel obtained from fossil fuel (Lele, 2006). Many countries including India have decided to blend 5-10% of *Jatropha* oil in fossil fuel used presently. Thus, the demand for elite seedlings of *Jatropha* is increasing day by day. The seedlings obtained from seeds are heterozygous and hence many a times do not give high yield potential. Tissue culture has become a versatile tool for propagation, preservation and genetic modification of such economically important plant species. It is reported by Basha and Sujatha (2007) that there are genetically divergent accessions of *J. curcas* L in India and hence, it is necessary to develop the suitable protocol for *in vitro* propagation of *J. curcas* L. Tissue culture technique on *J.curcas* L. has been employed by Sujatha *et al.*, (2005), Shilpa and Amla (2007), Meenakshi and Sarika (2008). Timir (2007) has reported on somatic embryogenesis in *J.curcas* L. There is a need to modify these protocols for further rapid multiplication of elite clones using different explants and with variation in media composition. *In vitro* techniques for induction of callus and somatic embryos are necessarily used for genetic manipulation (Junaid *et al.*, 2006). Such genetic modifications are required to induce pest and disease resistance and to improve the oil productivity of this tree species. The *Jatropha* plants in Gulbarga area have shown prevalenve of pest attack fungal disease and chlorotic leaves causing severe growth reduction. Many times the seed filling is not proper, producing immature and wrinkled seeds. Thus, the situation demands innovative approaches to improve the productivity of *Jatropha* for achieving desirable objectives. Present investigation was carried out to develop *in vitro* technology for callus induction, proliferation, somatic embryogenesis and shoot bud induction for semiarid climatic condition area of Gulbarga, Karnataka, India.

MATERIAL AND METHODS

Healthy young explants of *J. curcas* was collected from Biodiesel Technology Park of Gulbarga University, Gulbarga, Young leaves, petioles, internodes and seeds were washed separately under running tap water. Explants were disinfected with serial immersions in soapy water (Tween 20) for approximately 5 min, followed by 0.1% $HgCl_2$ (w/v) for 3 min and followed by 4% $NaOCl_2$ v/v for 5 min and finally rinsed with sterile distilled water. Explants were cut into appropriate sizes (leaf and petiole explants 10 mm^2, internodes for 7-15 mm^2, cotyledon and endosperm 10mm^2) using sterile forceps and knife under laminar air flow.

Culture media : For callus induction leaf and petiole were transformed on MS medium supplemented with different levels of plant growth regulators as detailed in Table 1. Similarly for the induction of somatic embryogenesis, cotyledon and endosperm explants were inoculated on MS medium supplemented with different levels of growth regulators as shown in Table 2. Shoot induction was achieved by culturing the internodes on MS medium supplemented with different

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levels of plant growth regulators as depicted in Table 3. The media were adjusted to pH 5.8 ± 0.2 and were solidified with 0.8% agar. They were sterilized at a pressure of 15lbs at 121°C for 20 min in an autoclave. All cultures were incubated at 25°C ± 2 with 16 hrs light/8 dark cycle under white fluorescent tubes. The experiments were performed in randomized design with 10 replicates were used for each treatment.

RESULTS AND DISCUSSION

Callus proliferation from leaf and petiole explants of *J. curcas* L. :

The surface sterilized explants leaf and petiole inoculated on MS salts medium supplements with a range of concentration of Plant growth regulators (PGRs). The effectiveness of PGRs for callus proliferation has been demonstrated for *J. curcas* L. (Rashida and Rabia, 2007). Callus obtained were friable, compact and morpghogenic. An excellent callus induction was observed at 0.93µM Kinetin and 0.73µM IBA (Fig 1b) in petiole explants than in leaf, This result is contrary to reports by Sujatha and Mukta (1996) who obtained higher callus from leaf explants.

Proliferation : To obtain callus proliferation, a portion of callus obtained from previous sets of explants was aseptically subcultured.

1. Effect of Auxin : Auxin like 2, 4-D were used alone respectively. Good callus growth was observed. These results are in agreement with those of Rashida and Rabia, (2007) who succeeded in callus induction in *J. curcas* L.

2. Combined effect of Auxin and Cytokinin : To study the combined effect of auxin and cytokinin on callus proliferation, three sets of experiments were conducted. 2, 4-D with Kinetin Kinetin with IBA and BAP with IAA. Good callus growth was observed in MS medium containing 0.93µM Kinetin and 0.73µM IBA. This results supports Sujatha and Mukta (1996), where combination of auxin and cytokinin enhanced the callus induction in *J. curcas* L.

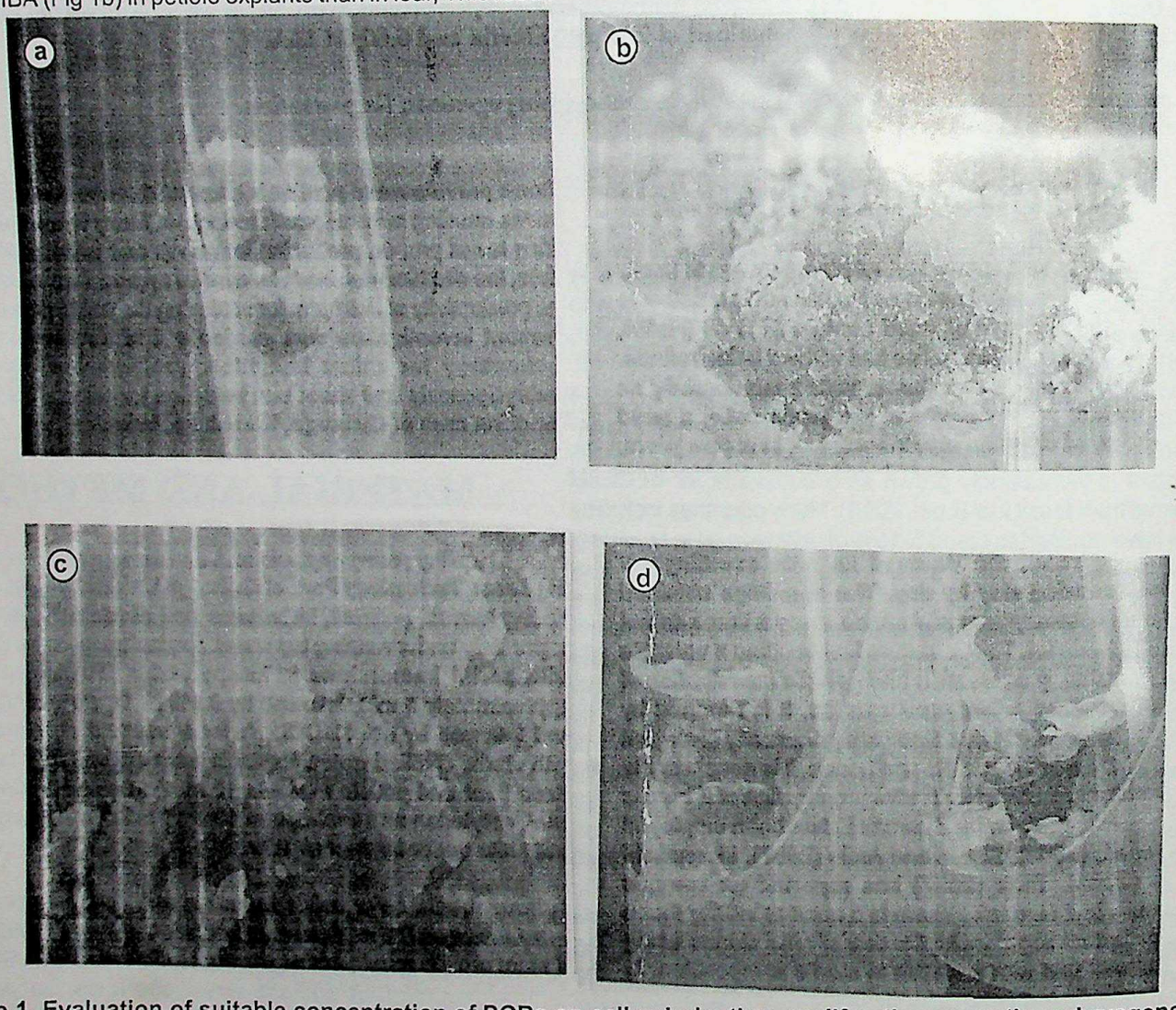

Figure 1. Evaluation of suitable concentration of PGRs on callus induction, proliferation, somatic embryogenesis and regeneration in *Jatropha curcas* L.
(a) Callus induction from petiole explants
(b) Callus proliferation obtained on MS medium + 0.93µM/l Kinetin and 0.73 µM/l BA
(c) Somatic embryogenesis on MS medium + 2.69µM/l NAA + 4.44µM/l BAP
(d) Shoots from internodes explants on MS medium + 2.32 µM/l Kinetin + 6.66 µM/l BAP

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Table 1 : Effects of 2, 4-D, Kinetin, IBA, BAP and IAA on callus induction in *J. curcas* L.

MS Media / Hormone (µM)					Frequency of callus %	Fresh weight (gm)	Name of Callus
2, 4-D	Kinetin	IBA	BAP	IAA			
1.36	-	-	-	-	30	0.06 ± 0.01	Friable Whitish
3.17	-	-	-	-	70	0.01 ± 0.01	Friable Whitish
4.52	-	-	-	-	70	0.41 ± 0.33	Friable Whitish
6.33	-	-	-	-	100	0.17 ± 0.00	Friable Whitish
9.05	-	-	-	-	60	0.04 ± 0.01	Friable Whitish
9.05	9.29	-	-	-	70	0.05 ± 0.02	Compact Watery
1.36	4.65	-	-	-	90	0.13 ± 0.01	Compact Watery
3.17	2.79	-	-	-	70	0.18 ± 0.06	Compact Watery
4.52	1.39	-	-	-	70	0.10 ± 0.02	Compact Watery
9.05	0.93	-	-	-	70	0.66 ± 0.28	Compact Watery
-	0.93	0.73	-	-	100	4.00 ± 0.49	Morphogenic Green
-	1.86	1.48	-	-	85	1.6 ± 0.23	Morphogenic Green
-	3.72	3.42	-	-	85	1.52 ± 0.16	Morphogenic Green
-	-	-	0.88	0.25	100	0.18 ± 0.01	Compact Watery
-	-	-	1.78	0.57	70	0.39 ± 0.25	Compact Watery
-	-	-	3.55	0.57	85	0.25 ± 0.22	Compact Watery

Results are from triplicates
Mean ± standard error from ANOVA program

Table 2 : Somatic embryogenesis from cotyledon and endosperm explants of *J. curcas* L.

MS Media/Hormones (µM/l)		No. of explants examined		Frequency of embryos per explants (%)		Fresh weight (gm)	
NAA	BAP	Cotyledon	Endosperm	Cotyledon	Endosperm	Cotyledon	Endosperm
2.69	0.22	15	15	60	80	0.58 ± 0.02	0.85 ± 0.01
2.69	0.44	15	15	60	80	0.53 ± 0.03	0.81 ± 0.04
2.69	2.22	15	15	70	80	0.81 ± 0.08	0.71 ± 0.08
2.69	4.44	15	15	60	100	0.69 ± 0.06	1.77 ± 0.14
2.69	8.87	15	15	70	80	0.55 ± 0.03	0.53 ± 0.07

Result are from triplicates
Mean ± standard error from ANOVA program

Table 3 : Effect of Kinetin and BAP on shoot formation in *J. curcas* L.

MS Media / Hormones (µM/l)		No. of shoots per plants	Shoot length (cm^2)	Survival of shoot %
KIN	BAP			
0.93	6.66	0.66 ± 2.08	7.3 ± 2.08	80
1.39	6.66	0.33 ± 0.33	6.5 ± 1.78	80
1.86	6.66	0.66 ± 0.00	6.9 ± 1.75	96
2.32	6.66	3.00 ± 0.00	8.0 ± 2.15	100
2.79	6.66	1.30 ± 0.33	7.0 ± 2.04	96

Results are from triplicates
Mean ± standard error from ANOVA program

Somatic embryogenesis from endosperm and cotyledonary explants of *J. curcas* L.

Cotyledonary and endosperm was used as explants for somatic embryogenesis. Endosperm responded well when compared with cotyledon explants. The data presented in Table 2 reports that the MS supplemented with 2.15µM NAA and 4.44µM BAP was found to be most suitable for the initiation and development of somatic embryo (Fig c). Kalimuthu *et al.*, (2007) has reported somatic embryogenesis from Cotyledonary explants. The present work on somatic embryogenesis shows that high concentration of cytokinin induces somatic embryo effectively in *J. curcas* explants and combination of auxin and cytokinin has influenced the frequency and maturation of somatic embryos.

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Table 4 : Types of explants and concentration of growth regulators used for tissue culture studies of *J. curcas* L.

MS Media / Hormone (mg/l)	Explants	Callus induction	Callus proliferation	Somatic embryogenesis	Shoot regeneration
MS + 2,4-D 6.33 µM/l	Leaf Petiole	+ ++	- +	- -	- -
MS + 2,4 D 1.36µM/l + KIN 4.65 µM/l	Leaf Petiole	+ +	- -	- -	- -
MS + Kinetin 0.93µM/l+ IBA 0.73µM/l	Leaf Petiole	+ +++	+ +++	- -	- +
MS + NAA 2.69µM/l+ KIN 4.44µM/l	Endosperm Cotyledon	+++ ++	+++ ++	+++ ++	- -
MS + Kinetin 2.32µM/l+ BAP 6.66µM/l	Internodes	+	-	-	+++

Direct shoot regeneration from internodes of *J. curcas* L.

Young internodes segments did not show any leaching or browning of tissues. The internodes cultured on MS basal medium with Kinetin and BAP showed varied response (Table 3). The best response obtained in the presence of 2.32µM Kinetin and 6.66µM BAP shoots induced per nodal explants. Sujatha and Mukta (1996) has reported higher regeneration from petiole explants in *J. curcas*. The beneficial effects of BAP for increasing shoot regeneration efficiency were reported in *Cajanus cajan* (George and Eapen, 1994). However, it has been observed in *Jatropha* that it requires high concentration of only one type of cytokinin (BAP) for induction phase and favors low concentration of another type of cytokinin (Kinetin) for escalation and proliferation of shoot cultures.

CONCLUSION

The paper reports that luxurious callus can be induced using petiole as explants on MS medium with 0.93µM Kinetin and 0.73µM IBA. Somatic embryogenesis was better with endosperm as explant on MS medium with 2.15µM NAA and 4.44µM BAP as PGRs. The MS medium supplemented with 2.32µM Kinetin and 6.66µM BAP was most suitable for inducing shoot. These protocols may be useful micropropagation of elite clones.

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Table 9 : Minimum inhibitry concentration of isolate to heavy metals.

Isolates	Resistance	MIC (µg/ml)
Bacillus spp	Iron	110
	Zinc	140
	Lead	150
	Magnesium	180
	Copper	180
Pseudomonas spp	Iron	200
	Zinc	250
	Lead	260
	Magnesium	280
	Copper	300
Azotobacter spp	Iron	120
	Zinc	130
	Lead	140
	Magnesium	140
	Copper	150
Glucanoacetobacter spp	Iron	60
	Zinc	80
	Lead	90
	Magnesium	90
	Copper	110
Phosphobacteria spp	Iron	60
	Zinc	60
	Lead	70
	Magnesium	80
	Copper	80
Aspergillus niger	Iron	60
	Zinc	80
	Lead	90
	Magnesium	120
	Copper	150
Penicillium spp	Iron	70
	Zinc	90
	Lead	100
	Magnesium	120
	Copper	130

130, 140, 140, 150µg/ml) by *Azotobacter* spp, (60, 80, 90, 90, 110 µg/ml) by *Glucanoacetobacter* spp, (60, 60, 70, 80, 80µg/ml) by *Phosphobacteria* spp, (60, 80, 90, 120, 150µg/ml) by *Aspergillus niger* and (70, 90, 100, 120 and 130µg/ml) by *Penicillium* spp respectively. Because the adsorption was selective (Nakajima and Akaguchi 1989) and high in the industrial waste waters having low ion concentration, it can be said that PGPR isolate can be used in tertiary treatment of Sugar mill effluent contaning heavy metals at low concentration (Vinita and Radhanath, 1992).

As a result, it can be concluded that PGPR isolate can be used in the removal or recovery of heavy metal ions, especially Iron ions from effluent. It can also be said that the usage of PGPR can be successful to remove Zinc, Lead, Magnesium and Copper element from the effluent containing high level of heavy metals. Further studies needed to increase the biosorption capacities of biomass and to develop appropriate technologies applicable in the treatment of effluent.

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Antimicrobial Efficiency of *Costus igneus* N.E.Br. (Costaceae)

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Costus igneus were subjected to soxhlet extraction using methanol, chloroform and petroleum ether solvents. The extracts were tested for the presence of various phytoconstituents and antibacterial activity by Agar well diffusion method. All the extracts have shown antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and Gram-nagative (*Escherichia coli*). The minimum inhibitory concentration was determined for each of the extract against the tested bacteria. It was found that the methanol extract was high inhibiting the tested bacteria followed by chloroform and petroleum ether extracts in most cases. Antifungal activity is carried out for *A. niger* and *C. keratinophilum* both of them showed moderate activity. The antimicrobial activity of solvent extracts of selected plants may be attributed to the presence of various phytochemicals present in them.

Keywords : Antibacterial activity, Methanol, Phytochemical, Bacteria, Fungi and *Costus*.

INTRODUCTION

Plants used in traditional medicine may constitute an important source of new biologically activity compounds. Utilization of plants for medicinal purposes in India has been documented in ancient literature (Ravindra and Vijay Kumar, 2007). The use of remedies from plant origin covers a wide veriety of maladies and constitutes an alternative way to antibiotics, Which is otherwise no longer promising due to the generalized antibiotic resistance among pathogenic microorganisms. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the microorganisms. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Phytomedicines derived from plants have shown great promise in the treatment of various diseases which includes viral infections (Bhagath *et al.*, 2008). *Costus igneus* N.E. Br. Common name fiery costus or spiral slag is an herbaceous belongs to Costaceae family. It is a succulent perennial herb, growing up to 2.7 m high and having an erect stem. The plant possesses horizontal rhizomatous rootstock. It generally grows luxuriantly on clay loam soil near inland forest under moderate shade. The plant propagates vegetatively through rhizomes or via seeds dispersed by birds. Rhizome is the plant part used as medicine. In ayurveda or via seeds dispersed by birds. Rhizome is the plant part acrid, cooling, aphrodisiac, purgative, antihelminthic, depurative, febrifuge, expectorant and tonic (Edward, 1999). The present work evaluating the phytochemical constituents and antimicrobial properties of *Costus igneus*.

MATERIAL AND METHODS

Collection and Extraction of plant material : *Costus igneus* N.E. Br. were collected from Lakkavali region of Chikmaglure district. Karnataka.The plant were authenticated in Department of Applied Botany. Jnanasahyadri, Shankaraghatta and voucher specimen (KU/AB/KSV/45) deposited in the Department for future reference. The plant materials were washed thoroughly 2-3 times with running tap water and once with sterile water, shade dried, powdered and used for extraction. The powdered plant material was extracted with solvents namely petroleum ether, chloroform and methanol. A known amount of powdered material (500gm) was subjected to soxhlet extraction and exhaustively extracted with respective solvents for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the desiccator (Kumaraswamy *et al.*, 2008 and Manjunatha *et al.*, 2006). The extracts were subjected to preliminary phytochemical screening to screen the presence of various secondary metabolites in the solvent extracts (Kokate, 2004, Roberts *et al.*, 1981 and Parekh & Chanda, 2007).

Preliminary screening of solvent extracts for antibacterial activity : Gram-positive (*Staphylococcus aureus* MTCC-902 and Gram-negative (*Escherichia coli* MTCC-405 and *Pseudomonas aeruginosa* MTCC-1934) bacteria's were used in the antibacterial study. The bacteria were procured from Institute of Microbial Technology, Chandigarh. The bacteria were screened for their sensitivity towards the plant extracts by Agar well diffusion method (Tepe *et al.*, 2004). In this method,

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24 hours old Muller-Hinton broth cultures of test bacteria were swabbed unifomly on solidified sterile Muller-Hinton agar plates using sterile cotton swab. Aseptically wells of 6 mm dia were done in the inoculated plates with the help of gel puncher. Different solvent extracts (1mg/ml to DMSO), Standard (Chloramphenicol and Tetracycline 1mg/ml) and Control (DMSO) were added into the respectively labeled wells. The plates were incubated at 37°C for 24 hours in upright position, after incubation zone of inhibition was recorded. The experiment was carried out in triplicated to get average reading.

Determination of Minimum inhibitory concentration (MIC) : The MIC of the solvent extracts against test bacteria was tested by Agar well diffusion method (Tepe *et al.*, 2004). The 24 hours old standardized Muller-Hinton broth cultures of test bacteria were swabbed uniformly on solidified sterile Muller-Hinton agar plates using sterile cotton swab. Wells of 6 mm dia were done in the inoculated plates with the help of gel puncher. Various dilutions of the plant extracts (10, 25, and 50µg/ml of DMSO) were added into the respectively labeled wells. The plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition was recorded. The least dilution among four was considered as the MIC. The experiment was carried out in triplications to get average reading.

Antifungal assay : Fungi namely *Aspergillus niger* (MTCC478), *Chrysosporium keratinophilum* (MTCC1367) were tested for their susceptibility to the solvent extracts. All these cultures were obtained from MTCC collection IMTECH (Institute of Microbial Technology), Chandigarh, India. The cultures were maintained at 4°C and subcultures frequently in respective media. The antifungal activity of plant extracts by Agar well diffusion method (Tepe *et al.*, 2004). In this method, spore suspension of test fungi, in 0.01% Tween 80 in saline, were swabbed uniformly on solidified sterile Sabouraud's dextrose agar plates using sterile cotton swab. Aseptically wells of 6 mm diameter were bored in the inoculated plates with the help of sterile gel puncher and the extract (10mg/ml of DMSO), Standard (fluconazole, 1mg/ml) and Control (DMSO) were added into the respectively labeled wells. The plates were incubated at 28°C for 72 hours in upright position and the zone of inhibition was recorded. The experiment was carried in triplicates to get average reading.

RESULTS AND DISCUSSION

The presence of various secondary metabolites in the methanol extract of plants selected for study is given in Table-1. The phytoconstituents namely Tannins, steroids and glycosides were found in the *Costus igneus* but flavonoids, alkaloids and saponins were absent.

The results of antimicrobial acitvity of solvent extracts were shown in Table – 2. Results show the presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of microbial growth and it was reported as positive and absence of zone as negative. It was found that all the solvent extracts of the plants *C. igneus* were found to inhibit growth of test microorganisms. Standard antibiotic Chloramphenicol (1mg/ml) for Gram+ve bacteria and Tetracycline (1mg/ml) for Gram - ve has shown inhibition of all tested bacteria and Flucanazole for Fungi. The control solvent DMSO did not revel inhibition of any of the tested bacteria.

Preliminary screening showed positive results for the antimicrobial activity, with the help of preliminary screening, minimal inhibitory concentrations (MIC) of extracts wree done. MIC of different solvents extracts was shown in Table-3. From the table it is clear that the methanol extracts of the plants are more effective in inhibiting test bacteria then others. The methanol extract of concentration 25 µg/ml was effective in inhibiting 50% of test bacteria. Following methanol extract, petroleum ether extract showed inhibition of test bacteria at low concentration when compared to chloroform extract in most cases. Among Gram-negative bacteria, *P.aeruginosa* has shown susceptibility at higher concentration (50µg/ml) then others.

MIC of different solvent extracts was shown in Table-4. Methanol extracts were found to possess more fungal activity followed by petroleum ether and chloroform extracts. Among tested fungi *A. niger* was showed more activity in methanol extract followed by *C. keratinophilum*. Chloroform extract of *C. igneus* was not found effective against *C. keratinophilum*. Previously reported that the rhizome of *Costus speciouse* as a constitutive principle with antifungal activity against *Cladosporium cucumerinum, Colletotricum, Curvularia* sp. and *Penicillium* sp. (Bandara *et al.*, 1988). It has been clearly exhibited in our study.

The antimycotic activity of extracts could be due to the presence of various phytoconstituents. Interest in plants with antimicrobial properties has reviewed as a result of current problems associated with the use of antibiotics. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005). In our study, the extracts have shown promising results in terms of inhibition of bacteria tested. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infections diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant (Fabricant and Farnsworth, 2001).

Table 1 : Phytochemical constituents in the methanol extracts of selected plants.

Phytochemical group	*C. igneus*
Tannins	+
Terpenoid	–
Alkaloid	–
Steroid	+
Saponins	–
Flavonoids	–
Glycosides	+

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Antimicrobial Efficiency of *Costus igneus* N.E.Br. (Costaceae)

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Costus igneus were subjected to soxhlet extraction using methanol, chloroform and petroleum ether solvents. The extracts were tested for the presence of various phytoconstituents and antibacterial activity by Agar well diffusion method. All the extracts have shown antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and Gram-nagative (*Escherichia coli*). The minimum inhibitory concentration was determined for each of the extract against the tested bacteria. It was found that the methanol extract was high inhibiting the tested bacteria followed by chloroform and petroleum ether extracts in most cases. Antifungal activity is carried out for *A. niger* and *C. keratinophilum* both of them showed moderate activity. The antimicrobial activity of solvent extracts of selected plants may be attributed to the presence of various phytochemicals present in them.

Keywords : Antibacterial activity, Methanol, Phytochemical, Bacteria, Fungi and *Costus*.

INTRODUCTION

Plants used in traditional medicine may constitute an important source of new biologically activity compounds. Utilization of plants for medicinal purposes in India has been documented in ancient literature (Ravindra and Vijay Kumar, 2007). The use of remedies from plant origin covers a wide veriety of maladies and constitutes an alternative way to antibiotics, Which is otherwise no longer promising due to the generalized antibiotic resistance among pathogenic microorganisms. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the microorganisms. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Phytomedicines derived from plants have shown great promise in the treatment of various diseases which includes viral infections (Bhagath *et al.*, 2008). *Costus igneus* N.E. Br. Common name fiery costus or spiral slag is an herbaceous belongs to Costaceae family. It is a succulent perennial herb, growing up to 2.7 m high and having an erect stem. The plant possesses horizontal rhizomatous rootstock. It generally grows luxuriantly on clay loam soil near inland forest under moderate shade. The plant propagates vegetatively through rhizomes or via seeds dispersed by birds. Rhizome is the plant part used as medicine. In ayurveda or via seeds dispersed by birds. Rhizome is the plant part acrid, cooling, aphrodisiac, purgative, antihelminthic, depurative, febrifuge, expectorant and tonic (Edward, 1999). The present work evaluating the phytochemical constituents and antimicrobial properties of *Costus igneus*.

MATERIAL AND METHODS

Collection and Extraction of plant material : *Costus igneus* N.E. Br. were collected from Lakkavali region of Chikmaglure district. Karnataka.The plant were authenticated in Department of Applied Botany. Jnanasahyadri, Shankaraghatta and voucher specimen (KU/AB/KSV/45) deposited in the Department for future reference. The plant materials were washed thoroughly 2-3 times with running tap water and once with sterile water, shade dried, powdered and used for extraction. The powdered plant material was extracted with solvents namely petroleum ether, chloroform and methanol. A known amount of powdered material (500gm) was subjected to soxhlet extraction and exhaustively extracted with respective solvents for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the desiccator (Kumaraswamy *et al.*, 2008 and Manjunatha *et al.*, 2006). The extracts were subjected to preliminary phytochemical screening to screen the presence of various secondary metabolites in the solvent extracts (Kokate, 2004, Roberts *et al.*, 1981 and Parekh & Chanda, 2007).

Preliminary screening of solvent extracts for antibacterial activity : Gram-positive (*Staphylococcus aureus* MTCC-902 and Gram-negative (*Escherichia coli* MTCC-405 and *Pseudomonas aeruginosa* MTCC-1934) bacteria's were used in the antibacterial study. The bacteria were procured from Institute of Microbial Technology, Chandigarh. The bacteria were screened for their sensitivity towards the plant extracts by Agar well diffusion method (Tepe *et al.*, 2004). In this method,

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24 hours old Muller-Hinton broth cultures of test bacteria were swabbed unifomly on solidified sterile Muller-Hinton agar plates using sterile cotton swab. Aseptically wells of 6 mm dia were done in the inoculated plates with the help of gel puncher. Different solvent extracts (1mg/ml to DMSO), Standard (Chloramphenicol and Tetracycline 1mg/ml) and Control (DMSO) were added into the respectively labeled wells. The plates were incubated at 37°C for 24 hours in upright position, after incubation zone of inhibition was recorded. The experiment was carried out in triplicated to get average reading.

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Antimicrobial and Antiplasmid Activities of Clove Extract and Eugenol

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Antimicrobial activity of cloves and eugenol was tested by agar-disc-diffusion method and MIC (minimum inhibitory concentration) by tube dilution method against non-pathogenic and resistant pathogenic gram-positive (*Staphylococcus aureus, Streptococcus pneumoniae, Bacillus cereus*) and gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris*) and fungi *Candida albicans, Aspergillus niger* and *Fusarium oxysporum*. Eugenol was more potential in killing bacteria and fungi than clove extract. The MIC of clove extract ranged between 12.5 - 25µg/ml and eugenol 0.2 - 1.0µg/ml for all the bacteria and fungi tested. Membrane leakage of UV_{260} (nucleic acid) and UV_{280} (proteins) absorbing material from bacteria into extracellular medium gradually increased after treating the bacteria with the test compound and proved that this may be one of the modes of bactericidal action. The time course of lethal action was also studied against *E. coli* and *Staphylococcus aureus*. Clove extract took 240 min and 210 min for effective (100%) killing of *E. coli* and *S. aureus* respectively where as eugenol took only 45min for effective killing of both the bacteria. Studies on elimination of β-lactamase enzyme activity and antiplasmid activity was done using *E. coli* HB101 but neither the clove extract nor the active principle (Eugenol) possess the property of eliminating either β-lactamase activity or drug resistant plasmid.

Keywords : Eugenol, MIC, β-lactamase, antiplasmid, antimicrobial etc.

INTRODUCTION

Cloves (*Syzygium aromaticum*, syn. *Eugenia aromaticum* or *Eugenia caryophillata*) are the aromatic dried flower buds of an ever green tree belong to the family Myrtaceae (Srivastava and Malhotra, 1991). In India, cloves are grown in Nilgiri, Tenkasi - hills and in Kanyakumari district of Tamilnadu state. It is also cultivated in Kottayam and Quilon districts of Kerala. Cloves are used in ayurveda, Chinese medicine and western herbalism. Cloves contain about 15 to 20% of volatile oil/essential oil, 10 to 13% tannins (gallatonic acid), resin, chromone and eugenin. The volatile oil of the clove contains 70 to 90% of eugenol, eugenyl acetate, caryophyllenes and small quantities of esters, ketones and alcohols (Chaieb *et al*, 2007).

Cloves are carminative (Phyllis and James, 2000), antioxidant (Lee and Shibamoto, 2001), antiulcerogenic (Bae *et al*, 1998) and antithrombotic (Srivastava and Malhotra, 1991).The volatile oil of clove eugenol is a strong germicide, a powerful antiseptic, a feeble local anesthetic applied to decayed teeth, and as a dental analgesic (Sashidhar, 2002), also used to treat acne, warts, and scars.

Clove oil is also an effective mosquito repellent (Trongliki *et al*. 2005), fragrant and flavouring agent (Chaieb *et al*, 2007). However, clove oil is toxic to human cells. If ingested or injected in sufficient quantity, may cause life threatening complications, including acute respiratory distress syndrome, hepatic failure and CNS disorders (Kirseh, 1990; Lane *et al* 1991). In the light of above information it was proposed to compare antimicrobial and antiplasmid properties of clove extract and eugenol, as plasmids are often responsible for drug resistance and resistance to many β-lactam antibiotics is often caused by β-lactamase enzyme.

MATERIAL AND METHODS

Plant material : Cloves were purchased from local market and Eugenol from Sigma chemicals ltd.

Bacterial cultures : Non-pathogenic Gram-positive bacteria - *Staphylococcus aureus* (NCIM -2079), *Bacillus cereus* (NCIM -2016) and *Streptococcus pneumoniae* (NCIM -2080).

Non-pathogenic Gram-negative bacteria - *Escherichia coli* (NCIM -2089), *Klebsiella pneumoniae* (NCIM -2957), and *Pseudomonas aeruginosa* (NCIM 2200), *E. coli* HB101 were obtained from NCIM, NCL, Pune.

Resistant pathogenic bacteria - *E. coli* - 1 was isolated from a patient's urine sample and *Staphylococcus aureus* - 1 from pus of an infected person.

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Fungal cultures : *Candida albicans, Aspergillus niger* and *Fusarium oxysporum* were obtained from Department of Botany, Osmania University, Hyderabad.

Chemicals and Media: Purchased from Qualigens and Hi-Media laboratories India ltd.

Extraction of Cloves : The cloves were ground to a fine powder before extraction. The Clove powder (50g) was then extracted with acetone using soxhlet apparatus by continuous heat extraction for 24hrs. The extract obtained was concentrated to dryness by evaporating the solvent under reduced pressure (Shastry *et al*, 2005). The concentrate thus obtained was dissolved in dimethyl sulfoxide (DMSO) such that the final concentration of the extract would be 1g/ml of DMSO.

Antimicrobial screening by disc diffusion method : The *in vitro* antimicrobial activity of acetone extract of clove and eugenol were carried out by disc diffusion method (Elgayyar *et al*, 2001). Actively growing log phase cultures were mixed in soft nutrient agar (Nutrient broth with 1% agar) and fungal spores in potato dextrose agar and plated. The 1µl of clove extract (1mg/1µl) and 1µl of eugenol were loaded onto different filter paper discs prepared from Whatmann No 1 filter paper. The discs were then placed on the agar medium containing the cultures, and incubated for 24hrs at 37°C for bacteria and at 28°C for 2 to 3 days for fungi. The diameter of zone of growth inhibition was recorded. The effects were compared with that of the standard antibacterial ampicillin (10µg/disc) and antifungal clotrimazole (10µg/disc). DMSO alone served as control.

Minimum inhibitory concentration : MIC was determined by serial dilution method (Stokes, 1975). Two fold serial dilution of the test compounds were carried out in the nutrient broth. To each test tube an inoculum containing 10^5 cells/ml of actively growing bacterial cultures in log phase was added. The culture tubes were incubated at 37°C for 24hrs. After the incubation, a loop full of treated cultures of bacteria are streaked on nutrient agar media and incubated for 18hrs at 37°C. The plates were checked for the growth of bacteria and MIC of the test compound was determined and expressed in µg/ml.

Determination of time course of lethal action on bacteria : *E.coli, Staphylococcus aureus* were treated with test compounds at MIC values and at regular time intervals aliquots of treated cultures were drawn, diluted appropriately and plated on nutrient agar plates. The inoculated plates were incubated at 37°C for 24hrs for colony development. The number of colonies were counted and the number of viable cells was determined.

Mode of action of clove extract and eugenol on bacteria : The method of Heipieper (1992) was followed to determine the leakage of UV_{260} and UV_{280} absorbing material. The cell suspension was prepared. *S. aureus* cells were grown overnight with continuous shaking in nutrient broth at 37°C, harvested, washed with 10mm EDTA and then twice in distilled water by centrifugation each time at 6000rpm for 15 minutes at 4°C and resuspended such that the absorbance of the final suspension was 2.0 at A_{450}. After incubation for 30mins at room temperature, the test clove extract and eugenol were added separately to suspension at MIC concentration. At regular intervals of time aliquots of the samples were drawn, centrifuged and UV_{260} and UV_{280} absorbing material in the supernatant was measured.

Test for development of resistance for bacteria against clove extract and Eugenol : Log phase culture of *E.coli* was exposed to ½ MIC of clove extract and eugenol for 18hrs at 37°C and then transferred to fresh medium containing ½ MIC of test compound. In this manner, the culture was passed 3 times and then the culture was streaked on nutrient agar media plates containing 1 MIC of test compound and then incubated for 24hrs at 37°C. If growth occurred it indicated that organism developed resistance and, if no growth occurred it indicated that no resistance was developed against test compounds.

Effect of test compound on β-lactamase enzyme activity: Few bacterial strains develop resistance against β-lactam drugs by producing an enzyme β-lactamase or pencillinase. This enzyme converts biologically active penicillin or ampicillin to an inactive penicilloic acid which adsorbs iodine. The adsorption or non-adsorption of iodine can be detected by starch indicator system. Inhibition of β-lactamase in bacteria (*E. coli* HB101) was studied as per the procedure of Odugbemi *et al.* (1977).

Antiplasmid activity : Test for elimination of plasmid by clove extract and eugenol was carried according to the procedure described by Winkler *et al.* (1979) was done in three steps. (1) Treatment of the drug resistant *E.coli* HB101 containing PBR322 plasmid responsible for Amp^r and Tet^r at ½ MIC of the test compound. (2) Extraction of plasmid from the treated and untreated bacteria cultures. (3) Visual confirmation (presence or loss) of plasmid by agarose-gel electrophoresis using UV transilluminator.

RESULTS AND DISCUSSION

The acetone clove extract and eugenol was studied for their antimicrobial properties *in vitro*. The disc diffusion method revealed that the MIC of the clove extract ranged between 12.5 - 25µg/ml for all the bacteria tested. *S. aureus* was found to be more sensitive (12.5µg/ml) than other Gram-positive bacteria, where as the entire Gram-negative bacteria tested were equally sensitive and the MIC was 25µg/ml. Eugenol is more efficient than clove extract; the MIC is 0.2 to 0.8µg/ml. *S.aureus* was more sensitive followed by *St.pneumoniae* and *B.cereus* (0.2, 0.6 and 0.8µg/ml respectively) among the Gram-negative bacteria, *Klebsiella pneumoniae* and *Proteus vulgaris* are sensitive followed by *E.coli* (0.4 & 0.8µg/ml respectively). The time course of lethal action studied against *E.coli* and *S.aureus* showed that clove extract took 240min & 210min for 100% killing of *E.coli* & *S.aureus* respectively and Eugenol took only 45min for 100% killing of both the bacteria (Fig 1 & 2). Membrane leakage of UV_{260} & UV_{280} absorbing material from *S.aureus* cells into extra cellular medium proved that this

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Table 1 : Antibacterial activity of clove extract and eugenol by disc-diffusion method.

S.No.	Micro organisms	Zone of growth inhibition in mm		
		Clove extract (1mg/µl)	Eugenol (1µl)	Ampicillin (10µg/disc)
1.	*Staphylococcus aureus*	19.0	20.0	22.0
2.	*Streptococcus pneumoniae*	12.3	14.5	13.0
3.	*Bacillus cereus*	11.7	14.0	19.0
4.	*Escherichia coli*	10.0	12.0	18.0
5.	*Klebsiella pneumoniae*	13.0	14.0	20.0
6.	*Pseudomonas aeruginosa*	12.8	18.0	24.0
7.	*Proteus vulgaris*	11.0	12.0	18.5
8.	*Escherichia coli* (urine)	9.0	11.0	8.0
9.	*Staphylococcus aureus* (pus)	16.0	20.0	NI

Table 2 : Antifungal activity of clove extract, eugenol and clotrimazole (for comparison) by disc-diffusion method.

S.No.	Micro organisms	Zone of growth inhibition in mm		
		Clove extract (2.5 mg)	Eugenol (1µl)	Clotrimazole (10µg/disc)
1.	*Candida albicans*	13.5	13	14
2.	*Aspergillus niger*	11.0	20	11
3.	*Fusarium*	12.5	21	10.3

Table 3 : Minimum inhibitory concentration of clove extract, eugenol and ampicillin (for comparison) in µg/ml.

S.No.	Micro organisms	Zone of growth inhibition in mm		
		Clove extract	Eugenol	Ampicillin
1.	*Staphylococcus aureus*	12.5	0.20	0.98
2.	*Streptococcus pneumoniae*	25.0	0.60	1.96
3.	*Bacillus cereus*	25.0	0.80	31.2
4.	*Escherichia coli*	25.0	0.80	1.96
5.	*Klebsiella pneumoniae*	25.0	0.40	0.98
6.	*Pseudomonas aeruginosa*	25.0	0.40	0.98
7.	*Proteus vulgaris*	25.0	0.80	31.2
8.	*Escherichia coli* (urine)	50.0	1.60	125
9.	*Staphylococcus aureus* (pus)	200.0	6.40	>250

may be one of the modes of lethal action. UV_{260} absorbing Nucleic acid material leakage gradually increased after 15min, but UV_{280} absorbing proteins leakage was initially slow but increased after 90min (Fig 3 & 4). Development of resistance by bacteria *E. coli* against both clove extract and Eugenol was interestingly not noticed even after 10 time's serial passage in ½ MIC of the test compounds. Both clove extract and Eugenol could not inhibit β-lactamase enzyme activity and eliminate drug resistant plasmid from the bacteria (Fig 5). The present study are in harmony to those reported by Burst & Reinders (2003) that clove oil was antibacterial (Lopez *et al*, 2005, Sashidar, 2002).

The present study and earlier reports reveals that cloves and the active principle of cloves, eugenol are effective antimicrobials. Findings from current study support the use of cloves in traditional medicine for the treatment of various bacterial and fungal infections and also local herbal or traditional medicinal industries modernizing their production systems.

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Fig.1: Time course of lethal action of clove extract and eugenol against *S.aureus*.

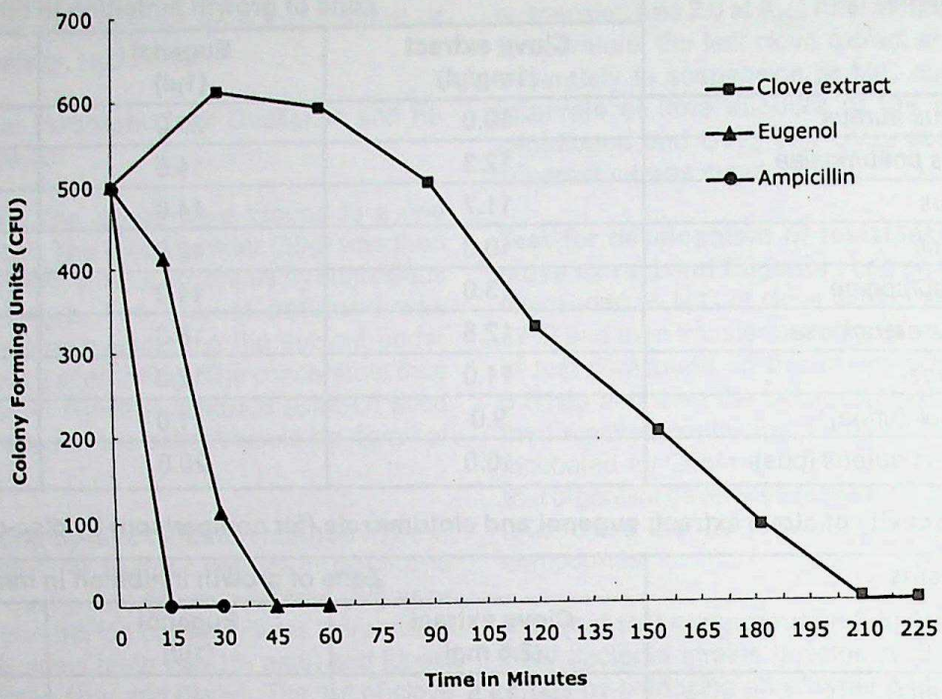

Fig. 2: Time Course of lethal action of clove extract and eugenol against *E.coli*.

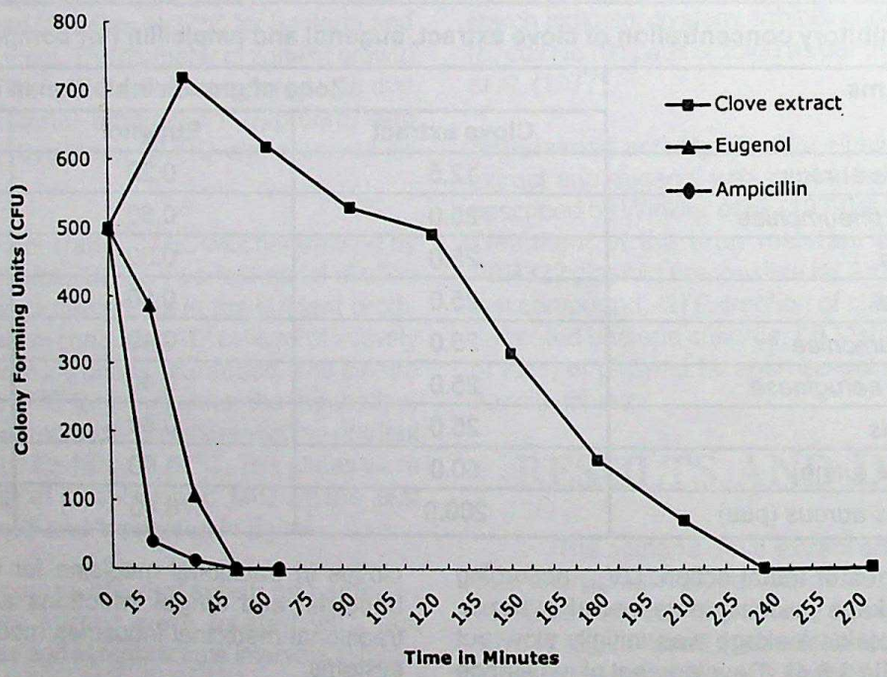

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Fig. 3: Effect of clove extract and eugenol on leakage of UV_{260} nm absorbing material from *S.aureus* cells at MIC.

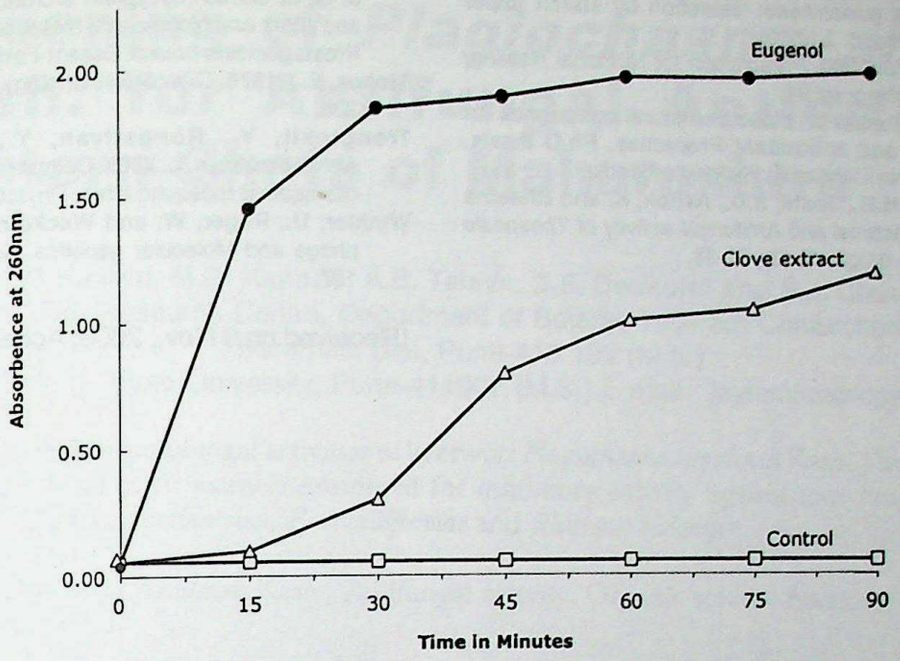

Fig. 4: Effect of clove extract and eugenol on leakage of UV_{280} nm absorbing material from *E. coli* cells at MIC.

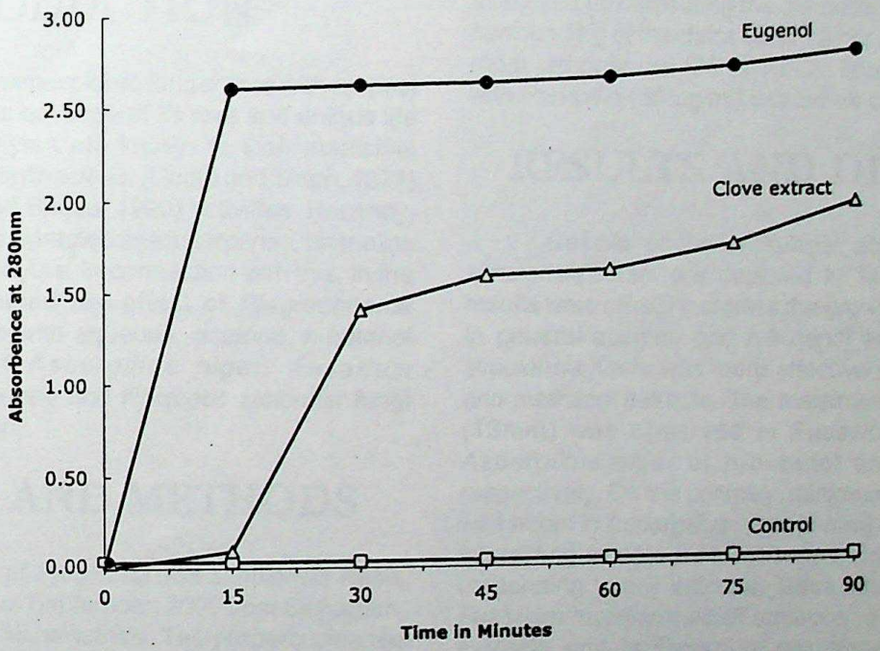

Fig. 5: Agarose gel electrophoresis bands in detection of plasmid after treatment with clove extract and Eugenol.

Lane 1 : Plasmid (1kb λ marker)

Lane 2 : Clove extract

Lane 3 : Eugenol

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Screening of *Plagiochasma simulensis* Kash. for Antifungal Activities from Western Ghats of Maharashtra

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Present work is depends on antifungal activities of liverwort *Plagiochasma simulensis* Kash. The aqueous, acetone, n-butanol and methanol plant extracts examined for inhibitory activity against four fungal pathogens like *Aspergillus niger, Fusarium graminearum, F. moniliformae* and *Rhizopus stolonifer.*

Keywords : *Plagiochasma simulensis* Kash., Antifungal activity, Organic solvent fractions, Western Ghats, Maharashtra.

INTRODUCTION

Bryophytes remain unexplored longer time with respect to antimicrobial properties because of its rare and unique life cycle. Number of bryophytes are known to their medicinal (Chang and Shao, 1986), antibacterial (Gupta and Singh, 1971) and antibiotic (Chopra and Bhatla, 1990) activities. Recently, Asakawa (1994 and 2001) extracted sesquiterpenes, phenolics and terpinoids from bryophytes. In connection with this, in the present study we examined the effect of *Plagiochasma simulensis* Kash. extracts with aqueous, acetone, n-butanol and methanol against *Aspergillus niger, Fusarium graminearum, F. moniliformae* and *Rhizopus stolonifer* fungi to prove antifungal activity.

MATERIAL AND METHODS

The plant material of *Plagiochasma simulensis* Kash. was collected in the month of September, 2009 from Sinhagarh, region of Western Ghats, Maharashtra. The properly cleaned (10 g) of plant material was homogenized with 10 ml distilled water as well as organic solvents like acetone, n-butanol, methanol separately. Homogenized material was shake on rotary shaker (250-300 rpm) for 24 hrs and filtered it with double layered muslin cloth and make 20 ml final volume with respective solutions (pH - 7). Filtrate was stored in refrigerator for further use in antifungal assays.

Antifungal properties of extracts were tested against the aforesaid fungi by filter paper disc diffusion method (Ronald *et al.*, 1984). Sterile Whatmann No. 1 paper discs (6 mm) were saturated with respective extracts then dried and placed on fungi inoculated Sabourd's agar plates. On each agar plate three filter paper discs were kept. Antifungal activity was assessed by measuring the diameter of growth inhibition zone surrounding of the discs up to 72 hrs at an interval of 24 hrs at room temperature (28 to 30°C). The results were compared with Penicillin (50 µg/ml) treated as control.

RESULTS AND DISCUSSION

Results of the antifungal activity of *Plagiochasma simulensis* Kash. are depicted in Table-1 and Plate-1. The results were clearly indicates the growth inhibition of test fungi. In general acetone and n-butanol extract of *Plagiochasma simulensis* Kash. was more effective as compare to aqueous and methanol extracts. The maximum growth inhibition zone (13mm) was observed in *Fusarium graminearum* and *Aspergillus niger* of n-butanol and methanol extracts respectively. On the contrary, minimum growth inhibition zone was noted in *Aspergillus niger* (6 mm) of aqueous extract. It is interesting to note that, *Rhizopus stolonifer* growth was not responding to any extracts. Trace inhibition was recorded in *Fusarium moniliformae* of aqueous, n-butanol and methanol extracts and in *Fusarium graminearum* of acetone and methanol extracts. All results were compared with Penicillin (50 µg/ml) as a control but growth inhibition of all test fungi was altogether absent in it.

Joshi *et al.* (1990) observed the effect of *Exermotheca tuberifera* Kash. extracts on 04 bacterial strains *viz., Escherichia coli, Klebsiella pneumonae, Bacillus substilis* and *Staphylococcus aureus* and reported growth inhibition of all test bacterial strains. The *in-vitro* antifungal activity of *Pallavicinea lyelli* was studied by Subhisha and Subramoniam (2005) against four test fungi (*Aspergillus niger, A. fumigatus, Fusarium oxysporum* and *Candida albicans*) using disc diffusion method. They concluded that water, alcohol and hexane extracts of *Pallavicinea lyelli* showed varying levels of

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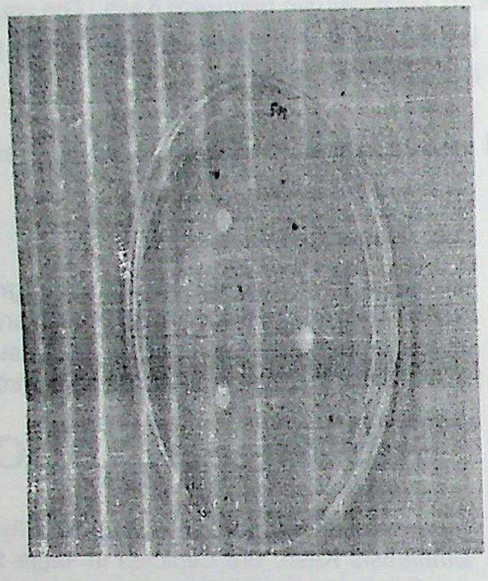
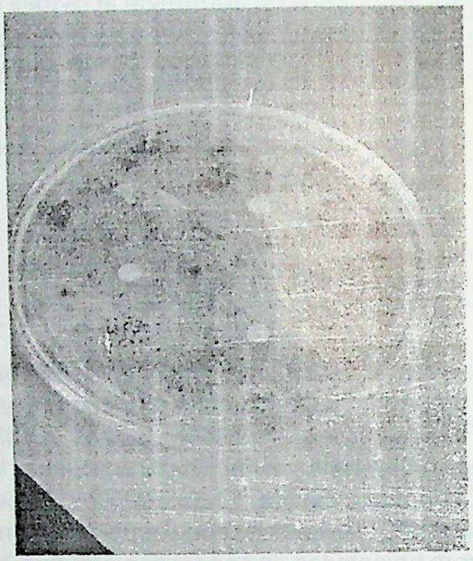
Table 1 : Antifungal screening of *Plagiochasma simulensis* Kash. against test fungi using disc diffusion method.

Nature of *Plagiochasma simulensis* Kash. extract	Diameter of inhibition zone of fungi (mm)			
	Rhizopus stolonifer	*Fusarium moniliformae*	*Fusarium graminearum*	*Aspergillus niger*
Control (Penicillin 50 µg/ml)	–	–	–	–
Aqueous	–	±	–	6
Acetone	–	8	±	9
n-butanol	–	±	13	9
Methanol	–	±	±	13

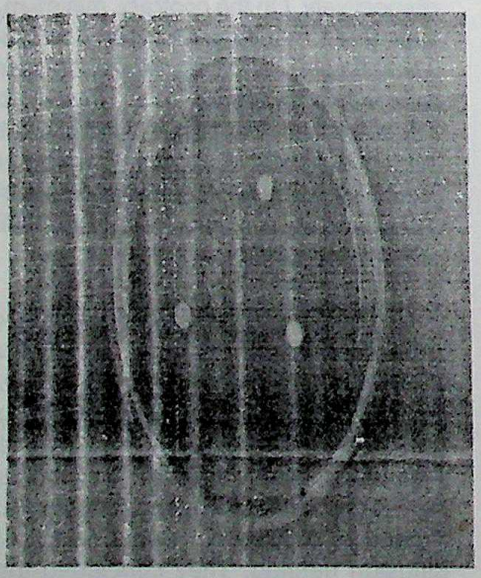
Plate 1 : Antifungal effect of *Plagiochasma simulensis* Kash. extracts against fungal strains.



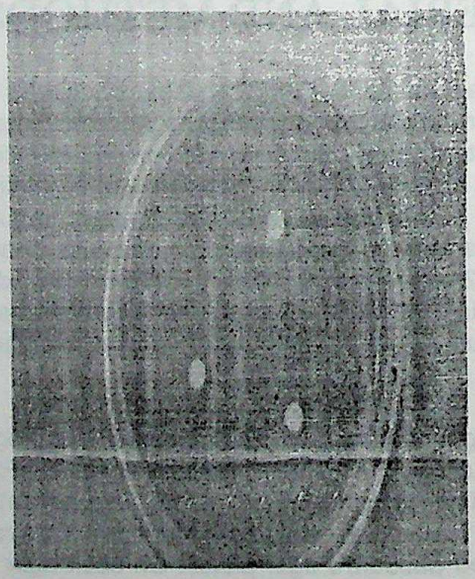
Fusarium moniliformae
(acetone extract)



Fusarium graminearum
(n-butanol extract)



Aspergillus niger
(n-butanol extract)



Aspergillus niger
(methanol extract)

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activity against the test fungi; the alcohol extract exhibited the maximum activity. Out of four fungi, *Aspergillus fumigatus* was found to be most sensitive.

Different liverworts and mosses screened to determine their antimicrobial activity against the selected bacteria and fungal pathogenic species were studied by Bodade *et al.* (2008) and recorded promising effect on growth inhibition of test organisms. Aqueous, methanol, n-propanol and petroleum ether extracts of 40 Cyanobacterial isolates belonging of nine genera were examined for inhibitory activity against five fungal pathogens (*viz., Aspergillus niger, A. flavus, Colletotrichum musae, Paecilomyces lilacinus* and *Fusarium oxysporum*) by Pawar and Puranik (2008).

The antimicrobial activity of bryophytes depends on the physiological age of the species, season of the collection and ecological conditions in which they are growing (Joshi *et al.*, 1990). Therefore variations are observed in such studies. In the present work we also find out the antifungal activity of *Plagiochasma simulensis* Kash. against four test fungi. It is hoped that this piece of work will unravel many intricate problems pertaining to antibiotic properties of liverworts and will also give a guideline to feature work.

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Evaluation of Suitable Concentration of PGRs on Callus Induction, Proliferation, Somatic Embryogenesis and Regeneration in *Jatropha curcas* L.

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The present study was undertaken to evaluate the most suitable concentration of growth regulators *viz*, 2, 4-D, IBA, BAP, Kinetin, IAA and NAA for callus induction, its proliferation, somatic embryogenesis and regeneration in explants of *Jatropha curcas* L. Good callus formation was obtained on MS medium containing 0.93µM Kinetin and 0.73 µM IBA. Excellent embryogenesis was observed at 2.15µM NAA and 4.44µM BAP from endosperm explants. Rapid *in vitro* propagation by shoot proliferation on internodal segments isolated from field grown mature plants was obtained at 2.32µM Kinetin and 6.66µM IBA.

Keywords : *Jatropha curcas,* Callus induction, Somatic embryogenesis, Regeneration.

INTRODUCTION

J. curcas L. a well known biofuel plant belongs to family Euphorbiaceae, commonly known as Physic nut is a native to America and Africa. *J. curcas* seed contain 37% oil (Heller, 1996). The oil can be combusted as fuel without being refined. It burns with clear smoke-free flame, tested successfully as fuel diesel engine. The by-products are press cake, a good organic fertilizer, oil contains also insecticide. In recent years, the plant is increasingly grown as a substitute for biodiesel obtained from fossil fuel (Lele, 2006). Many countries including India have decided to blend 5-10% of *Jatropha* oil in fossil fuel used presently. Thus, the demand for elite seedlings of *Jatropha* is increasing day by day. The seedlings obtained from seeds are heterozygous and hence many a times do not give high yield potential. Tissue culture has become a versatile tool for propagation, preservation and genetic modification of such economically important plant species. It is reported by Basha and Sujatha (2007) that there are genetically divergent accessions of *J. curcas* L in India and hence, it is necessary to develop the suitable protocol for *in vitro* propagation of *J. curcas* L. Tissue culture technique on *J.curcas* L. has been employed by Sujatha *et al.,* (2005), Shilpa and Amla (2007), Meenakshi and Sarika (2008). Timir (2007) has reported on somatic embryogenesis in *J.curcas* L. There is a need to modify these protocols for further rapid multiplication of elite clones using different explants and with variation in media composition. *In vitro* techniques for induction of callus and somatic embryos are necessarily used for genetic manipulation (Junaid *et al.,* 2006). Such genetic modifications are required to induce pest and disease resistance and to improve the oil productivity of this tree species. The *Jatropha* plants in Gulbarga area have shown prevalenve of pest attack fungal disease and chlorotic leaves causing severe growth reduction. Many times the seed filling is not proper, producing immature and wrinkled seeds. Thus, the situation demands innovative approaches to improve the productivity of *Jatropha* for achieving desirable objectives. Present investigation was carried out to develop *in vitro* technology for callus induction, proliferation, somatic embryogenesis and shoot bud induction for semiarid climatic condition area of Gulbarga, Karnataka, India.

MATERIAL AND METHODS

Healthy young explants of *J. curcas* was collected from Biodiesel Technology Park of Gulbarga University, Gulbarga, Young leaves, petioles, internodes and seeds were washed separately under running tap water. Explants were disinfected with serial immersions in soapy water (Tween 20) for approximately 5 min, followed by 0.1% $HgCl_2$ (w/v) for 3 min and followed by 4% $NaOCl_2$ v/v for 5 min and finally rinsed with sterile distilled water. Explants were cut into appropriate sizes (leaf and petiole explants 10 mm^2, internodes for 7-15 mm^2, cotyledon and endosperm 10mm^2) using sterile forceps and knife under laminar air flow.

Culture media : For callus induction leaf and petiole were transformed on MS medium supplemented with different levels of plant growth regulators as detailed in Table 1. Similarly for the induction of somatic embryogenesis, cotyledon and endosperm explants were inoculated on MS medium supplemented with different levels of growth regulators as shown in Table 2. Shoot induction was achieved by culturing the internodes on MS medium supplemented with different

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levels of plant growth regulators as depicted in Table 3. The media were adjusted to pH 5.8 ± 0.2 and were solidified with 0.8% agar. They were sterilized at a pressure of 15lbs at 121°C for 20 min in an autoclave. All cultures were incubated at 25°C ± 2 with 16 hrs light/8 dark cycle under white fluorescent tubes. The experiments were performed in randomized design with 10 replicates were used for each treatment.

RESULTS AND DISCUSSION

Callus proliferation from leaf and petiole explants of *J. curcas* L. :

The surface sterilized explants leaf and petiole inoculated on MS salts medium supplements with a range of concentration of Plant growth regulators (PGRs). The effectiveness of PGRs for callus proliferation has been demonstrated for *J. curcas* L. (Rashida and Rabia, 2007). Callus obtained were friable, compact and morpghogenic. An excellent callus induction was observed at 0.93μM Kinetin and 0.73μM IBA (Fig 1b) in petiole explants than in leaf, This result is contrary to reports by Sujatha and Mukta (1996) who obtained higher callus from leaf explants.

Proliferation : To obtain callus proliferation, a portion of callus obtained from previous sets of explants was aseptically subcultured.

1. Effect of Auxin : Auxin like 2, 4-D were used alone respectively. Good callus growth was observed. These results are in agreement with those of Rashida and Rabia, (2007) who succeeded in callus induction in *J. curcas* L.

2. Combined effect of Auxin and Cytokinin : To study the combined effect of auxin and cytokinin on callus proliferation, three sets of experiments were conducted. 2, 4-D with Kinetin Kinetin with IBA and BAP with IAA. Good callus growth was observed in MS medium containing 0.93μM Kinetin and 0.73μM IBA. This results supports Sujatha and Mukta (1996), where combination of auxin and cytokinin enhanced the callus induction in *J. curcas* L.

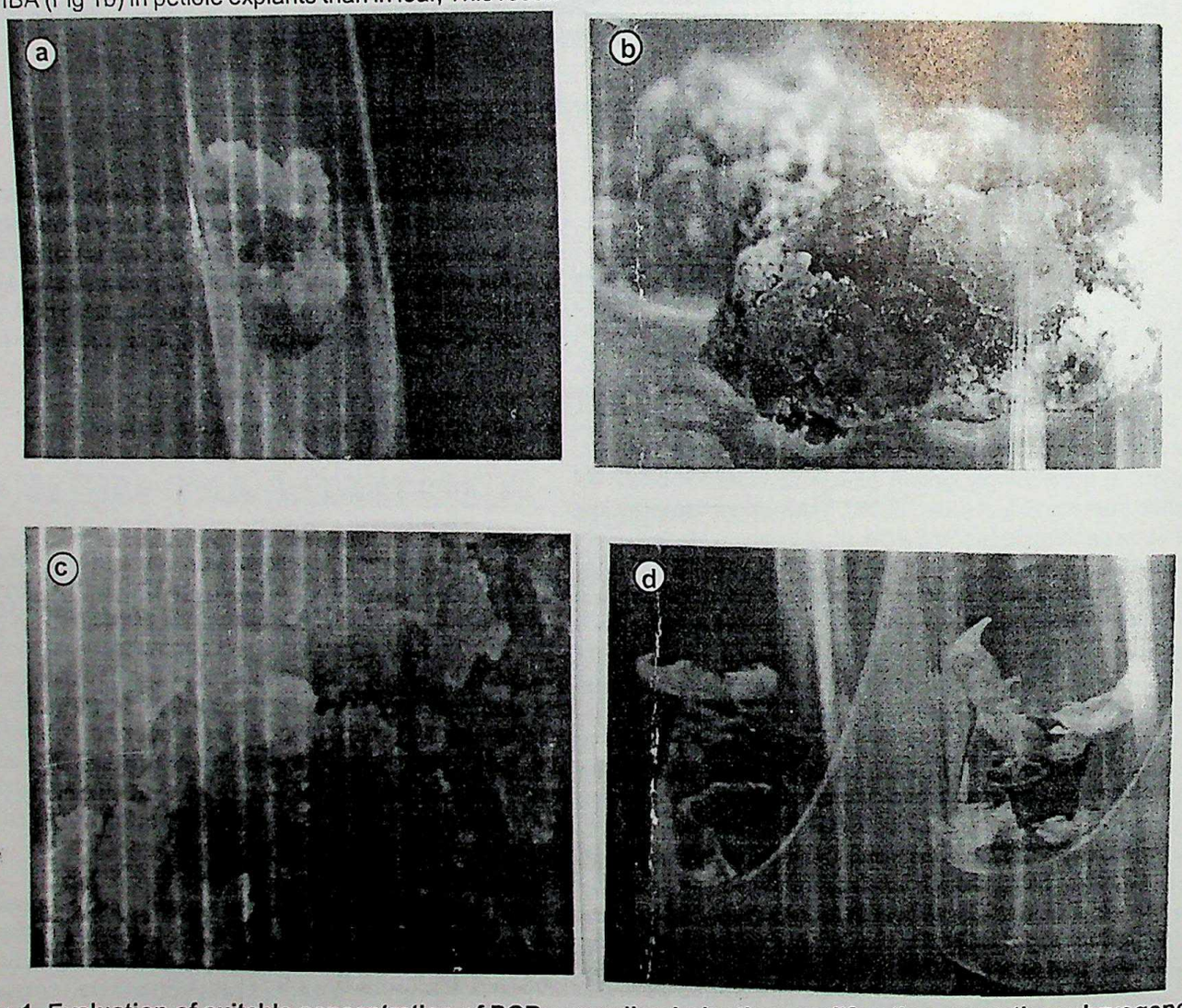

Figure 1. Evaluation of suitable concentration of PGRs on callus induction, proliferation, somatic embryogenesis and regeneration in *Jatropha curcas* L.
(a) Callus induction from petiole explants
(b) Callus proliferation obtained on MS medium + 0.93μM/l Kinetin and 0.73 μM/l BA
(c) Somatic embryogenesis on MS medium + 2.69μM/l NAA + 4.44μM/l BAP
(d) Shoots from internodes explants on MS medium + 2.32 μM/l Kinetin + 6.66 μM/l BAP

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Table 1 : Effects of 2, 4-D, Kinetin, IBA, BAP and IAA on callus induction in *J. curcas* L.

MS Media / Hormone (µM)					Frequency of callus %	Fresh weight (gm)	Name of Callus
2, 4-D	Kinetin	IBA	BAP	IAA			
1.36	-	-	-	-	30	0.06 ± 0.01	Friable Whitish
3.17	-	-	-	-	70	0.01 ± 0.01	Friable Whitish
4.52	-	-	-	-	70	0.41 ± 0.33	Friable Whitish
6.33	-	-	-	-	100	0.17 ± 0.00	Friable Whitish
9.05	-	-	-	-	60	0.04 ± 0.01	Friable Whitish
9.05	9.29	-	-	-	70	0.05 ± 0.02	Compact Watery
1.36	4.65	-	-	-	90	0.13 ± 0.01	Compact Watery
3.17	2.79	-	-	-	70	0.18 ± 0.06	Compact Watery
4.52	1.39	-	-	-	70	0.10 ± 0.02	Compact Watery
9.05	0.93	-	-	-	70	0.66 ± 0.28	Compact Watery
-	0.93	0.73	-	-	100	4.00 ± 0.49	Morphogenic Green
-	1.86	1.48	-	-	85	1.6 ± 0.23	Morphogenic Green
-	3.72	3.42	-	-	85	1.52 ± 0.16	Morphogenic Green
-	-	-	0.88	0.25	100	0.18 ± 0.01	Compact Watery
-	-	-	1.78	0.57	70	0.39 ± 0.25	Compact Watery
-	-	-	3.55	0.57	85	0.25 ± 0.22	Compact Watery

Results are from triplicates
Mean ± standard error from ANOVA program

Table 2 : Somatic embryogenesis from cotyledon and endosperm explants of *J. curcas* L.

MS Media/Hormones (µM/l)		No. of explants examined		Frequency of embryos per explants (%)		Fresh weight (gm)	
NAA	BAP	Cotyledon	Endosperm	Cotyledon	Endosperm	Cotyledon	Endosperm
2.69	0.22	15	15	60	80	0.58 ± 0.02	0.85 ± 0.01
2.69	0.44	15	15	60	80	0.53 ± 0.03	0.81 ± 0.04
2.69	2.22	15	15	70	80	0.81 ± 0.08	0.71 ± 0.08
2.69	4.44	15	15	60	100	0.69 ± 0.06	1.77 ± 0.14
2.69	8.87	15	15	70	80	0.55 ± 0.03	0.53 ± 0.07

Result are from triplicates
Mean ± standard error from ANOVA program

Table 3 : Effect of Kinetin and BAP on shoot formation in *J. curcas* L.

MS Media / Hormones (µM/l)		No. of shoots per plants	Shoot length (cm^2)	Survival of shoot %
KIN	BAP			
0.93	6.66	0.66 ± 2.08	7.3 ± 2.08	80
1.39	6.66	0.33 ± 0.33	6.5 ± 1.78	80
1.86	6.66	0.66 ± 0.00	6.9 ± 1.75	96
2.32	6.66	3.00 ± 0.00	8.0 ± 2.15	100
2.79	6.66	1.30 ± 0.33	7.0 ± 2.04	96

Results are from triplicates
Mean ± standard error from ANOVA program

Somatic embryogenesis from endosperm and cotyledonary explants of *J. curcas* L.

Cotyledonary and endosperm was used as explants for somatic embryogenesis. Endosperm responded well when compared with cotyledon explants. The data presented in Table 2 reports that the MS supplemented with 2.15µM NAA and 4.44µM BAP was found to be most suitable for the initiation and development of somatic embryo (Fig c). Kalimuthu *et al.*, (2007) has reported somatic embryogenesis from Cotyledonary explants. The present work on somatic embryogenesis shows that high concentration of cytokinin induces somatic embryo effectively in *J. curcas* explants and combination of auxin and cytokinin has influenced the frequency and maturation of somatic embryos.

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Table 4 : Types of explants and concentration of growth regulators used for tissue culture studies of *J. curcas* L.

MS Media / Hormone (mg/l)	Explants	Callus induction	Callus proliferation	Somatic embryogenesis	Shoot regeneration
MS + 2,4-D 6.33 µM/l	Leaf Petiole	+ ++	- +	- -	- -
MS + 2,4 D 1.36µM/l + KIN 4.65 µM/l	Leaf Petiole	+ +	- -	- -	- -
MS + Kinetin 0.93µM/l+ IBA 0.73µM/l	Leaf Petiole	+ +++	+ +++	- -	- +
MS + NAA 2.69µM/l+ KIN 4.44µM/l	Endosperm Cotyledon	+++ ++	+++ ++	+++ ++	- -
MS + Kinetin 2.32µM/l+ BAP 6.66µM/l	Internodes	+	-	-	+++

Direct shoot regeneration from internodes of *J. curcas* L.

Young internodes segments did not show any leaching or browning of tissues. The internodes cultured on MS basal medium with Kinetin and BAP showed varied response (Table 3). The best response obtained in the presence of 2.32µM Kinetin and 6.66µM BAP shoots induced per nodal explants. Sujatha and Mukta (1996) has reported higher regeneration from petiole explants in *J. curcas*. The beneficial effects of BAP for increasing shoot regeneration efficiency were reported in *Cajanus cajan* (George and Eapen, 1994). However, it has been observed in *Jatropha* that it requires high concentration of only one type of cytokinin (BAP) for induction phase and favors low concentration of another type of cytokinin (Kinetin) for escalation and proliferation of shoot cultures.

CONCLUSION

The paper reports that luxurious callus can be induced using petiole as explants on MS medium with 0.93µM Kinetin and 0.73µM IBA. Somatic embryogenesis was better with endosperm as explant on MS medium with 2.15µM NAA and 4.44µM BAP as PGRs. The MS medium supplemented with 2.32µM Kinetin and 6.66µM BAP was most suitable for inducing shoot. These protocols may be useful micropropagation of elite clones.

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Preliminary Phytochemical Investigation and Antimicrobial Activity of *Sphaeranthus indicus* Linn.

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Leaves, flower stem and roots of *Sphaeranthus indicus* were extracted separately with methanol, ethanol, chloroform, petroleum ether and hot water and the extracts were screened for its phytochemical constituents. The plant revealed the presence of alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, cardiac glycosides, aminoacids, monosaccharides and reducing sugar. Leaves extracts showed significant amount of phytochemicals and hence antimicrobial studies of leaves extracts were carried out against bacterial species like *Bacillus* sp. *Staphylococcus* sp., *Klebsiella* sp., *E. coli, Pseudomonas,* sp., using filter paper and agar well diffusion method at 4 different concentrations. Methanolic and aqueous of *S. indicus* leaf extract showed the highest inhibitory effect compared to all other extracts and it showed good inhibitory activity against *Bacillus* sp., followed by *Staphylococcus* sp., The Gram-positive bacteria were found to be more susceptible than Gram-negative becteria. Antifungal activity of method and ethanol extracts were tested against *Penicillum* sp and *Aspergillus* sp. and the growth was found to be decreased with increasing concentration of the extracts.

Keywords : *Sphaeranthus indicus*, plant extract, phytochemical analysis, antibacterial activity, agar diffusion.

INTRODUCTION

The use of plants and plant producrts as medicine can be traced as far back as the beginning of human civilization. Medicinal plants are the richest bio–resource of drugs of traditional system of medicine, modern medicine, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediate and lead compounds in synthetic drugs (Ncube *et al.*, 2008). Phytochemicals are non–nutritive plant chemicals that are more comlex and specific and exert their action by resembling endogenous metabolites. ligands, hormones, signal transduction molecules or neurotransmitters due to similarities in their target sites. These plant based natural constitutents can be derived from any part of the plant like bark, leaves, flowers, roots, fruit and seeds, etc. According to world health organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections.

Sphaeranthus indicus belongs to the family Asteraceae. This herb is used as a laxative, digestible, demulcent, emollient and pectoral. All parts of the plant find medicinal uses. It acts as a good remedy for anaemica. hemicranias, leucoderma, bronchitis, insanity, tuberculosis, elephantiasis, spleen diseases, pain of the uterus and vagina, piles, asthma, boils, scabies, ringworms. It also treats leprosy, fever, filariasis, gout, hepatitis (Dwivedi *et al.,* 2008) and jaundice. Leaves are extremely useful in epilepsy, mental debility as a nerve tonic and antisyphilitic. The plant also found to possess macrofilaricidal, antidiabetic, anxiolytic and mosquito larvicidal effect. The juice of this plant is taken internally to retain pregnancy and to blacken grey hair. The extract of this plant also showed significant antibacterial, antifungal, antitumourous properties. In the present study, the phytochemical and antimicrobial properties of *S. indicus* were evaluated for its in therapeutic prepartions.

MATERIAL AND METHODS

Collection of plant materials and extraction : The plant material was collected from the rice fields of Manikandam, Tiruchirappalli district, Tamil Nadu in the month of January, 2009. The herbarium specimen was deposited in Rapinat Herbarium, St. Joseph's College, Trichy, Tamil Nadu, India.

The collected plant was washed thoroughly in tap water, shade dried and leaves, flower stems and roots of the plant were separated and coarsely powdered by a mechanical grinder. About 10 gm of each dried powder of the plant were soaked separately in 100 ml of different solvents like methanol, ethanol, chloroform and petroleum ether in conical flasks and

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then subjected to agitation on a rotary shaker for three days at 190–220 rpm. After 72 hrs. the plant extracts were filtered through Whatmann no. 42 paper separately and they were allowed to be concentrated by heating on a boiling water bath. Concentrated extracts were then preserved in labelled sterilized air tight bottles at 4°C in refrigerator until when required for use. Aqueous extract of the plant was prepared by soaking 10 gm of dried powder in 100ml of boiling water and the extract was also collected as descirbed earlier.

Leaves, flower stems and roots extract of different solvents were analysed for the presence of alkaloids, saponins, tannins, phenols, steriods, cardiac glycosides, anthraqunions, flavonoids, terpernoids, aminoacids, reducing sugars and monosaccharides as described by Soffowara (1994) and Siddiqui and Ali (1997).

Screening for alkaloids : Five ml of the extract was boiled with 2N HCl (5 ml) and mixture was filtered and to the filterate a few drops of Mayer's reagent was added. A cream colour precipitate produced immediately indicates the presence of alkaloids.

Screening for saponins : About 5 ml of the extract was boiled in 10 ml of distilled water in a test tube and the test tube was shaken vigorously for about 30 sec and allowed to stand for half an hour. Formation of froth indicates the presence of saponins.

Screening for tannins : To five ml of the extract, a few drops of 1% lead acetate were added. A yellow precipitate was formed, which indicates the presence of tannins.

Screening for phenols : About 2 ml of the extract was added to 2 ml of ferric chloride solution and appearance of deep bluish green colour solution indicates the presence of phenols.

Screening for steroids : One ml of the extract was dissolved in 10 ml of chloroform and equal volume of conc. Sulphuric acid was added by the sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence which indicates the presence of steroids.

Screening for cardiac glycosides : To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid were added and observed for the reddish brown at the junction of tow layers and the bluish green colour in the upper layer shows the presence of cardiac glycosides.

Screening for anthraquinones : About 5 ml of the extract was boiled with 10 ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Screening for flavonoids : To one ml of the extract, a few drops of dilute sodium hydroxide were added. An intense yellow colour was produced in the plant extract, which became colourless on addition of a few drops of dilute acid. This indicates the presence of flavonoids.

Screening for terpenoids : The extract was dissolved in 1 ml of chloroform, 1 ml of acetic anhydride was added following the addition of 2 ml of conc. H_2SO_4. Formation of reddish violet colour indicates the presence of terpenoids.

Screening for aminoacids : One ml of the extract was treated with few drops of Ninhydrin reagent. Appearance of purple colour shows the presence of aminoacids.

Screening for reducing sugars : To 1 ml of the extract, 5-8 drops to Fehling's solution was added and boiled and observed for the brick red precipitate.

Screening for monosaccharides : To 1 ml of the extract, 1 ml of Barfoed's reagent was added and heated on a water bath. Formation of brown precipitate indicates the presence of monosaccharides.

Test organisms : Microbial cultures were obtained from the Department of microbiology, Cauvery College of women, Trichy. Among five bacterial species investigated two Gram-positive bacteria were *Bacillus* sp. and *Staphylcoccus* sp. While three Gram-negative bacteria were *Pseudomonas* sp., *Klebsiella* sp., and *E.coli.* The fungal species used included *Aspergillus* sp., and *Penicillium* sp., All the organisms were carefully identified using standard microbiological methods. All the bacterial and fungal species were maintained at 4°C nutrient agar and Potato Dextrose agar slants respectively.

Antimicrobial assay : The crude extracts (methanol, ethanol, chloroform, petroleum ether and hot water) obtained from the leaves of *S.indicus* were studied for its antibacterial activity using agar well and filter paper disc diffusion methods at four different concentrations *i.e.,* 10µl, 20µl, 30µl, 40µl. Twenty four hours old cultures of the organisms to be tested were used. The Mueller Hinton agar plants were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. In agar well diffusion method, four holes measuring 5.0 mm each in diameter were made in the plants using a sterile cork borer. Various concentrations of the plant extracts were transferred into the holes and the plates were allowed to stand for one hour for pre-diffusion of the extracts to occur. The plates were then incubated at 37°C for 24 hr. At the end of incubation the plants were collected and the zones of inhibition that developed were measured. Triplicates were maintained and the averages of the zones of inhibition were calculated. In filter paper dise method, discs with 6 mm diameter were prepared using Whatmann filter paper and sterilized by autoclaving. Then, the discs had been impregnated with 10 ml of test extracts and introduced onto upper layer of the seeded agar plates.

Simultaneously, Gentamycin was used as a positive control and Dimethyl sulfoxide was used as a negative control. Methanol and ethanol extracts of the leaves were tested for its

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antifungal activity using streak plate method. Different concentratiion of each extract were introduced into 20 ml of PDA media and the content was mixed thoroughly and poured into the plates and allowed to solidify. After solidification,continous streaking of fungal inoculum was carrried out and the plates were incubated at 25°C for 42 hrs. At the end of incubation period, the fungal growth in all plate was compared for its decrease in growth.

RESULTS

Table 1 showed the phytochemical constituents of different extracts of the leaves and it revealed the presence of tannins, phenols, saponins, flavonoids, aminoacids, monosaccharides and reducing sugars. Methonal extracts of leaves showed the presence of alkaloids and the ethanolic extract contained cardiac glycosides. Terpenoids was absent in all the extracts. Among the various extracts, Methonal extracts showed the presence of significant amount of phytochemicals followed by aqueous and ethanol extract and this may contribute to better antibacterial activity. Leaves extracts showed significantly high amount of phytochemicals when compared to flower stem and root extracts. Hence in our study, leaves extracts were investigated for its antimicrobial activity.

Table 2 showed the phytochemical constituents of different extracts of the flower and they found to contain tannins, saponins, steroids, aminoacids, flavonoids, monosaccharides and reducing sugar. Terpenoids was present in aqueous extract and chloroform extracts. Similar to leaves extracts, significant amount of phytochemicals were seen in aqueous extracts of flower.

Table 3 showed the phytochemical constituents of different extracts of the root and they revealed the presence of tannins, phenols, saponins, steroids, aminoacids, terpenoids, reducing sugars, monosaccharides, aminoacids and flavonoids. Alkaloids and glycosides were absent in all the extracts of the plant. Among the various extracts, methanol and ethanol extracts showed the presence of significant amount of phytochemicals when compared to aqueous extracts.

Table 4 showed the antibacterial activity of leaves extracts of *S. indicus* against the test organisms using agar well diffusion method. Among the test organisms used in the study, Gram-positive bacteria showed more inhibition effect than Garm-negative bacteria and especially, *Bacillus* sp. showed higher zone of inhibition than *Staphylococcus* sp. Among the Gram-negative bacteria, *E. coli* showed better activity than *Klebsiella* sp. All the extracts did not exhibit any inhibitrory effect on *Pseudomonas* sp. The methanol extracts possess more antimicrobial activity when compared to aqueous and ethanol extracts. inhibition zone was increased with increase in concentration of the extract and thus exhibiting concentration.

In filter paper disc diffusion method, methanol, ethanol and water extracts showed considerable effect on *Bacillus* sp., *Staphylococcus* sp., and *Klebsiella* sp., but no effect on *E. coli* and *Pseudomonas sp.*, Chloroform and petroleum ether extract did not not show any inhibitory effect against all the organisms used for the study. *Sphaeranthus inducus* has notable antifungal activity against two fungal species. The growth of both *Aspergillus* sp., and *Penicillium* sp., were found to be decreased with increasing concentration of methanol and aqueous extracts of the leaves.

DISCUSSION

From the above result, the plant extracts found to possess better antimicrobial activity on the Gram–positive bacteria than Gram–negative bacteria and it may be due to the fast that Gram–negative bacteria possess an outer membrane, which acts as a barrier to many environmental substances including antibiotics. Similar antibacterial activity was observed in other plants of some family (Salie *et al.*, 1996). In most cases, alkaloids (Rahman *et al.*, 1997), flavonoids (Jayaprakasam *et al.*, 1999) and triterpenoids (Jain *et al.*, 2001) prove to be most important groups of compounds that showed wide range of antimicrobial activity. A sesquiterpene lactone, 7–hydroxyfrullanolide isolated from *S. indicus* had antimicrobial activity (Perumalsamy *et al.*, 1999). Steroids like β–sitosterol and stigmasterol is very common chemical constituents with gastroprotective activity in several ulcer models in rats. The terpenoids have also deen shwon to decrease blood sugar level in animal studies. Glycosides, flavonoids, tannins and alkaloids have hypoglycemic effects. The steroids and saponins are responsible for normal activites of central nervous system. Tannins act as antimicrobial agent by inhibiting extracellular enzymes, deprivating the substrates required for microbial growth or by inhibiting oxidative phosphorylation of microbial metabolism.

The result of the presnt study suggested that the plant extracts of *S. indicus* possess phytochemical compounds with significant antimicrobial activity. The methanolic and aqueous extracts showed good antibacterial activity when compared to all other solvents, and it may due to the presence of more phytochemical and hence, it can be used in therapeutic preparations.

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Table 1 : Phytochemical Screening of leaf extracts of *Sphaeranthus indicus* Linn.

Phytochemicals	Methanol	Ethanol	Chloroform	Petroleum Ether	Water
Tannins	+++	++	–	–	+++
Phenols	+++	+	–	–	+
Saponins	+++	–	–	–	++
Alkaloid	++	–	–	–	++
Flavonoids	++	–	–	–	++
Anthraquinones	–	–	–	–	–
Aminoacids	++	+	+	–	+++
Monosaccharides	+	+	–	–	+
Reducing sugar	+	+	–	–	+
Terpenoids	–	–	–	–	–
Cardiac glycosides	–	+	+	–	–
Steroids	+	++	+	+	–

(+++) – strongly present (+) – poorly present
(++) – moderately present (–) – absent

Table 2 : Phytochemical Screening of flower extracts of *Sphaeranthus indicus* Linn.

Phytochemicals	Methanol	Ethanol	Chloroform	Petroleum Ether	Water
Tannins	++	+	–	–	++
Phenols	++	+	–	–	++
Saponins	+	–	–	–	+
Alkaloid	–	–	–	–	+
Flavonoids	+	–	–	–	–
Anthraquinones	++	–	–	–	–
Aminoacids	+++	–	–	–	+
Monosaccharides	+++	+	–	–	+
Reducing sugar	–	+	–	–	+
Terpenoids	–	–	+	–	–
Cardiac glycosides	–	–	–	–	–
Steroids	+	+	–	+	–

(+++) – strongly present (+) – poorly present
(++) – moderately present (–) – absent

Table 3 : Phytochemical Screening of stem extracts of *Sphaeranthus indicus* Linn.

Phytochemicals	Methanol	Ethanol	Chloroform	Petroleum Ether	Water
Tannins	+	–	–	–	+
Phenols	–	–	–	–	–
Saponins	–	–	–	–	+
Alkaloid	–	–	–	–	–
Flavonoids	–	–	–	–	+
Anthraquinones	–	–	–	–	–
Aminoacids	++	+	–	–	++
Monosaccharides	++	+	–	–	+
Reducing sugar	+	+	–	–	+
Terpenoids	–	–	–	–	+
Cardiac glycosides	–	+	–	–	–
Steroids	+	++	+	–	–

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Table 4 : Phytochemical Screening of root extracts of *Sphaeranthus indicus* Linn.

Phytochemicals	Methanol	Ethanol	Chloroform	Petroleum Ether	Water
Tannins	+	+	–	–	+
Phenols	–	–	–	–	–
Saponins	–	–	–	–	+
Alkaloid	–	–	–	–	–
Flavonoids	+	+	–	–	+
Anthraquinones	–	–	–	–	–
Aminoacids	+	+	–	–	–
Monosaccharides	+	+	–	–	+
Reducing sugar	+	+	–	–	+
Terpenoids	+	+	–	–	+
Cardiac glycosides	–	–	–	–	–
Steroids	+	+	+	–	–

(+++) – strongly present (+) – poorly present (++) – moderately present (–) – absent

Table 5 : Antibacterial activity of leaf extracts of *Sphaeranthus indicus* Linn.

Extracts	Concentration (µl)	Zone of inhibition in mm				
		Bacillus sp.,	*Staphylococcus* sp.,	*Klebsiella* sp.,	*E. coli*	*Pseudomonas* sp.,
Water	10	1.5	1.5	2.5	0.5	–
	20	2.5	2	4	2	–
	30	3	3	5.5	3	–
	40	4.5	4	6	3.5	–
Ethanol	10	2	1.5	–	1.5	–
	20	2.5	2.5	3	2	–
	30	3.5	3	3.5	2.5	–
	40	4.5	4	4.5	3.5	–
Chloroform	10	–	1.5	–	1.5	–
	20	2	2	–	3	–
	30	4	3.5	–	4	–
	40	5	4.5	–	5	–
Petroleum ether	10	–	–	–	–	–
	20	–	–	–	–	–
	30	2	1.5	–	1.5	–
	40	3	2.5	–	2.5	–
Methanol	10	3	6	7	2	–
	20	4	7	8	3.5	–
	30	4.5	9.5	8.5	4	–
	40	5	10	9	5	–
Gentamycin		7	7	3	4	5

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Effect of Seed Inoculation of Plant Growth Promoting Rhizobacteria *Bacillus* (Producing Indole Acetic Acid) on Seedling Growth in Chickpea

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Out of 30 bacterial isolates obtained from the rhizosphere samples collected, from chickpea, 17 were selected based on Gram staining, cultural and biochemical characteristics. Only five *Bacillus* isolates and a standard strain MRS33 showed indole acetic acid production using Salkowski's reagent. three *Bacillus* isolates CBS79, CBS83, CBS92 produced significant amount of IAA (23.4 to 31.5 µg/ml supernatant) in Luria Bertani medium supplemented with tryptophan (100 µg/ml) after 4 days incubation. Thirteen *Bacillus* isolates *viz*, CBS21, CBS43, CBS50, CBS51, CBS58, CBS60, CBS62, CBS66, CBS71, CBS73, CBS80, CBS92, CBS93 and standard strain MRS33 also inhibited the growth of common fungal pathogen *Fusarium oxysporum*. Five *Bacillus* isolates, selected on the basis of IAA production were used as seed inoculants for root and shoot elongation studies of chickpea on plain water agar plates. Different *Bacillus* isolates showed initial stunting effect on plumule and radical development with subsequent stimulation of root and shoot growth by some *Bacillus* isolates under aseptic conditions. Most of the *Bacillus* strains showed stunting effect at 5 and 10 days in chickpea.

Keywords : Rhizobacteria *Bacillus*, IAA, Chickpea.

INTRODUCTION

Bacillus are a common PGPR found in the rhizosphere of cereal and legume crops. They have immense potential to be used as biofertilizer, biocontrol agent and/or in bioremediation due to their plant growth-promoting ability, antagonistic activity and degradation of pollutants in the contaminated or polluted soils.

No higher plant or animal is able to assimilate the molecular N_2 present in the atmosphere. But a number of microoorganisms can do this. Legumes have been found to utilize atmospheric N_2 depending upon the bacteria present in the nodules[17].

Subsequent studies have led to the identification of various sps. belonging the genera *Rhizobium, Clostridium, Azotobacter, Azospirillum* as an important N_2 fixing organisms. These microorganisms may be free living or in symbiotic associations with plants. The beneficial free living soil bacteria are referred as plant growth promoting *rhizobacteria* (PGPR) and are found in the *rhizosphere* of many different plants[21]. PGPR have been found to increase the growth of plant[29,36]. The rate of seedling emergence has been found to be increased by the application of PGPR[12,22,25]. A subset of PGPR strains also enhances the nodulation of legumes by *rhizobia* and are called as nodulation promoting *rhizobacteria* (NPR). PGPR promotes the plant growth by providing the plants with the compounds synthesized by itself or facilitating the uptake of nutrients from environment[14]. The plants growth promoting traits of the bacteria are many such as production of IAA, ammonia, HCN, siderophere, phosphate solabilization, antifungal activity[2],13. Several strains of *Pseudomonas, Azotobacter, Bacillus* and *Rhizobium* has been reported to produce IAA Auxin has been implicated in influencing many functions such as initiation of lateral and adventitious roots[23] in stimulation of cell division[9,19], elongation of root[35].

MATERIAL AND METHODS

Cultures, media, host plant species and growth condition: Standard cultures of and *Bacillus* sp. used in this study *Bacillus* strains were isolated and maintained on Luria Bertani (LB) Medium[30] and Nutrient Agar (NA) medium[8] Seeds of chickpea (*Cicer arietinum* L.) cultivar C235 (resistant to blight and susceptible to wilt) was used as host plant species.

Interactions of *Bacillus* sp. with fungal pathogen : *Bacillus* sp. isolates were screened for their interaction with pathogenic fungi *Fusarium oxysporum*, by spot test method using nutrient

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agar and potato dextrose agar (PDA) media.Spore suspension of *Fusarium oxysporum*, was prepared in 0.85% sterilized saline from 7 days old growth of fungus on PDA plates and 0.1 ml of this spore suspension was spread over the NA media plates. *Bacillus* spp. were spot inoculated onto these media plates and inhibition zones of fungal growth around the spotted growth were recorded after incubation for 5-7 days at 37 ± 1°C.

Detection of IAA production : IAA production in different *Bacillus* isolates was determined using Salkowski reagent[16] Auxin concentration values were obtained by preparing standard curve from IAA solution (0-100 µg ml^{-1}) and amount of IAA produced is expressed as µg IAA secreted per unit of optical density.

Intrinsic antibiotic resistance pattern of *Bacillus* strains : Intrinsic antibiotic resistance pattern of selected *Bacillus* strains was determined on LB plates containing the different concentrations of antibiotics, Filter sterilized stock solutions of antibiotics were added in LB molten agar kept at 60°C in water bath. The medium was gently shaken after adding the desired concentration of antibiotic and plated out. After solidification of the medium, the *Bacillus* strains were spot inoculated onto plates and incubated at 37 ± 1°C for 2-3 days. Control plates without antibiotics were also kept for comparison of growth. On the basis of appearance of growth on antibiotic plates, the antibiotic resistance pattern for each strain was determined.

Effect of *Bacillus* strains on seed germination and seedling growth of chickpea : Healthy seeds of chickpea (*Cicer arietinum* L.) cultivar C235 were surface sterilized with acidic alcohol (H_2SO_4 : ethanol, 7:3, v\v) for 3 min followed by thorough washing with repeated changes of sterilized distilled water[10]. The surface sterilized seeds were inoculated with broth culture of the *Bacillus* strains and its mutants for about 30 min. Inoculated seeds were kept for germination on plain water agar (0.8%) plates at 37±1°C under controlled conditions. Uninoculated seeds treated with LB broth were sown in plates as control. Effect of inoculation on root and shoot length was measured at 5 and 10 days after sowing.

RESULTS

The samples were then added to a hot water bath at 80°C and samples were dilution plated on NA medium plates. 30 presumptive *Bacillus* colonies were picked up and then were plated on starch agar plate, incubated at 37°C for 24 hours. White, uneven, spreading colonies were observed which indicated the *Bacillus* species. Test for catalase production and starch digestion were conducted. Finally 17 isolates displayed the positive activity further all isolates were Gram +ve and showed motility in hanging drop preparations using normal saline.

Screening *Bacillus* isolates for indole acetic acid production and antifungal activity : All the selected *Bacillus* isolates were initially screened for IAA production. *Bacillus* isolates were grown in Luria Bertani broth (4.5 ml) supplemented with tryptophan (100 µg\ml) at 37 ± 1°C for 24 h. In the growth suspension, equal amount of Salkowski's reagent (0.5 M $FeCl_3$ in 35% perchloric acid) was added and incubated at room temperature for 30 min. The change in colour from light yellow to pinkish or red indicated the production of indole acetic acid by the particular *Bacillus* isolate. *Bacillus* isolates CBS 30, CBS 79, CBS 83, CBS 87, CBS 92 and standard strain MRS 33 were found to produce indole-acetic acid in LB broth (Table 1). However, the intensity of colour change varied depending upon the amount of IAA produced by a particular isolate. *Bacillus* isolates *i.e.,* CBS 43, CBS 60, CBS 62, CBS 71, CBS 73, CBS 80, CBS 93, CBS 92, CBS 21, CBS 50, CBS 51, CBS 58, CBS 66 and standard strain MRS 33 inhibited the growth of *Fusarium oxysporum* in spot test on nutrient agar plates (Table 1). The diameter of inhibition zones (devoid of mycelial growth) varied with *Bacillus* isolates and larger zones of inhibition were observed with *Bacillus* isolates CBS 66 and standard strain MRS 33.

Production of indole acetic acid by different *Bacillus* isolates : Five IAA-producing *Bacillus* isolates were screened for quantitative analysis of indole-acetic acid produced using Salkowski's reagent. The amount of IAA produced varied from 11.4 to 28.9 µg/ml of supernatant (having 1.0 optical density) in different *Bacillus* isolates at 2 days culture (Table 2). At 4 days of culture growth, the amount of IAA (12.0 to 31.5 µg/ml) released in the supernatant increased. Maximum amount of IAA production was observed in *Bacillus* isolates CBS 92 at both 2 days and 4 days of culture growth.

Antibiotic resistance pattern of *Bacillus* strains. The selected *Bacillus* strains were spot inoculated onto LB plates supplemented with different antibiotics at a concentration from 20 to 50 µg/ml. The plates were incubated at 37 ± 1°C for 2-3 days. Control plates without antibiotics were also kept for growth comparisons. Strains CBS 30, MRS 33 showed resistance to five antibiotics (Table 3). All *Bacillus* strains grew on LB plates supplemented with antibiotics ampicillin.

Effect of seed inoculation with *Bacillus* isolates on root and shoot elongation in chickpea : Five *Bacillus* isolates were selected based on their ability to produce indole acetic acid and inhibition of fungal growth. These isolates were used as seed inoculations to study the effect on root and shoot elongation on plain water agar plates (0.8%) using seeds of chickpea cv. C235.

All the *Bacillus* strains showed stunting effect on root and shoot growth at 5 days in chickpea (Table 4). Maximum stunting effect on root as well as shoot growth was observed at 5 days with the strain CBS 79 and standard strain MRS 33. Strain CBS 87 showed stunting effect on shoot at both the stages of observations. All the *Bacillus* strains comparatively increased the root growth at 10 days than to 5 days observation.

DISCUSSION

A large number of microorganisms are found in plant rhizosphere. The microorganisms interact with each other and with plant in a way that may result in symbiotic, associative,

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neutralistic or detrimental effects depending upon the type of microorganisms involved, nutrient status in soil, abiotic and biotic soil environment and the plant defense system[6]. Out of 30 bacterial isolates obtained initially from chickpea and green gram rhizosphere, 17 isolates were finally selected as presumptive *Bacillus*, based on Gram staining and standard cultural characteristics. These isolates were first screened for indole acetic acid production and antifungal activity against common fungal pathogen *Fusarium oxysporum*. Out of 17 isolates tested, only five *Bacillus* isolates CBS 30, CBS 79, CBS 83, CBS 87, CBS 92 and standard strain MRS 33 were found to produce indole acetic acid (Table 2), The amount of IAA produced varied from 11.4 to 28.9 µg/ml of supernatant in different *Bacillus* isolates at 2 days of growth (Table 2). Three *Bacillus* isolates CBS 79, CBS 83, CBS 92 produced significant amount of IAA (12.0 to 31.5 ug/ml supernatant) at 4 days of growth. Production of plant growth hormones particularly IAA has long been considerd as an important attribute of PGPR strains and different bacterial strains have been found to produce IAA in varying amounts[26,32].

Barea *et al.* (1976) found that among 50 bacterial isolates obtained from the rhizosphere of various plants, 86,58 and 90% isolates produced auxins, gibberellins and kinetin-like substances, respectively. Auxin-like compounds were higher in rhizosphere soil of maize than in root-free soil, especially during seedling emergence[28], high levels of IAA (76.6 µm) were exerted by four deleterious rhizobacteria (*Micrococcus luteus, Streptoverticillium* sp., *Pseudomonas putida* and *Gluconobacter* sp.)". Lower amount of IAA (16.4 µm) were secreted by PGPR isolates including *Agrobacterium* sp., *Alcaligens piechaudii* and *Comamonas acidovorans*. Production of auxins by microbial isolates has been found to vary greatly among different species and stage and availability of substrates, strains and influenced by culture conditions, growth.

Various *Bacillus* isolates showed antifungal activities and inhibited the growth of common fungal pathogen *Fusarium oxysporum* (Table 1). Thus PGPR isolates call indirectly promote plant growth by suppressing the proliferation of disease-causing fungal pathogens. Several bacterial genera have shown their potential for biocontrol both under *in vivo* and *in vitro* conditions. Important among these biocontrol agents are *Agrobacterium*[20], *Pseudomonas*[15,27], *Bacillus*[1]. Use of such antagonistic rhizobacteria leading to promotion of plant growth is associated with suppression of growth of root borne fungal pathogens like *Fusarium* spp., *Pythium ultimum, Gaeumannomyces graminis*[3,11].

Attempts to control plants pathogenic fungi by antibiotic-producing or siderophore-producing rhizobacteria have met with variable success[18,31,33,34]. Competition for nutrients and infection sites between antagonistics bacteria and pathogens may also play an important role in action of biocontrol agent towards plant pathogens. Therefore, production of antibiotics or siderophores by *Bacillus* at the first stage of plant colonization may provide a selective advantage in the rhizosphere establishment because the growth of competitors for nutrients (including pathogens) could be suppressed[6].

Five *Bacillus* isolates selected on the basis of their ability to produce indole-acetic acid were used as seed inoculants to study their effect on root and shoot elongation on plain water agar plates (0.8%) using seeds of chickpea cv. C235. Most of the *Bacillus* strains showed stunting effect on root and shoot growth at 5 and 10 days in chickpea (Table 4).

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Table 1 : Production of Indole Acetic Acid and growth inhibition of *Fusarium oxysporum* by *Bacillus* isolates.

Rhizosphere sample	Isolate code No.	IAA production	Growth inhibition of *F. oxysporum*
Chickpea	CBS 21, CBS 43, CBS 50, CBS 51, CBS 58, CBS 60, CBS 62, CBS 71, CBS 73, CBS 80, CBS 93,	–	+
Chickpea	CBS 79, CBS 83, CBS 87	++	–
Chickpea	CBS 30	+	–
Chickpea	CBS 66	±	++++
Chickpea	CBS 92	+	+
Standard strain	MRS 33	±	+++

– = A lack of IAA production and lack of fungal growth inhibition
± = Low amount of IAA production and less growth inhibition
+ = Shows production of IAA and growth inhibition
>+ = Shows significant IAA production and bigger zone of fungal growth inhibition

Table 2 : Production of Indole Acetic Acid by different *Bacillus* isolates.

Bacillus culture	IAA production (µg/ml supernatant)	
	2 days	4 days
Control	0.0	0.0
CBS 30	11.4	12.0
CBS 79	19.4	24.0
CBS 83	19.3	23.4
CBS 87	19.6	20.9
CBS 92	28.9	31.5
MRS 33	14.4	20.7

Control contains LB broth and the Salkowski regent. IAA production was calculated on the basis of equal 1.0 optical density of bacterial growth suspension

Table 3 : Antibiotic resistance pattern of different *Bacillus* sp.

Bacillus strain	Control	Cm	Sm	Km	Nm	Tmp	Nal	Rif	Te	Amp	Spec
CBS 30	+	+	+	–	–	+	–	–	–	+	+
CBS 79	+	–	–	–	–	–	–	–	–	+	–
CBS 83	+	–	–	–	–	–	–	–	–	+	–
CBS 87	+	–	–	–	–	–	–	–	+	+	–
CBS 92	+	–	–	–	–	–	–	–	+	+	–
MRS 33	+	+	–	–	–	+	–	+	–	+	+

Cm = Chloramphenicol, Sm = Streptomycin, Km = Kanamycin, Nm = Neomycin, Nal = Nalidixic acid, Rif = Rifampicin, Te = Tetracyalin, Amp = Ampicilin, Tmp = Trimethoprim, Spec = Spectinomycin

Table 4 : Effect of *Bacillus* isolates on seedlings growth of chickpea.

Treatment	Root length (cm)		Shoot length (cm)	
	5 days	10 days	5 days	10 days
Control	15.60 ± 0.44	18.37 ± 0.82	9.27 ± 0.44	11.65 ± 1.1
CBS 30	13.71 ± 0.50	18.21 ± 0.50	9.13 ± 0.41	10.63 ± 0.85
CBS 79	10.70 ± 0.51	17.15 ± 0.75	7.55 ± 0.33	11.55 ± 1.1
CBS 83	11.11 ± 0.51	16.95 ± 0.51	8.21 ± 0.56	11.65 ± 1.9
CBS 87	10.75 ± 0.55	15.49 ± 0.05	8.89 ± 0.30	9.19 ± 1.0
CBS 92	12.77 ± 0.33	17.85 ± 0.29	9.35 ± 0.29	10.95 ± 1.7
MRS 33	10.29 ± 0.45	17.99 ± 0.20	7.45 ± 0.20	12.39 ± 2.2

Data are average values of five replicates means ± SE

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Use of Organic Manure and Microbial Inoculants for Production of Qualitative Containerised Seedlings of *Cedrus deodara* and *Cupressus torulosa*

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The containerised seedling production technology, especially use of polypacks / root trainers is dependent on suitable growing media for raising quality seedlings. In the present investigation, experiments were carried out to find out the suitable potting mixture for raising *Cedrus deodara* and *Cupressus torulosa* seedlings. Out of the eight media used M_6 (Forest Soil: Sand: *Pisolinthus tinctorius*) supported maximum survival of (75.05 and 78.40 per cent), plant height(14.60 and 17.46 cm), collar diameter (1.96 and 1.99 mm), shoot to root ratio (3.82 and 3.92), plant dry weight g/plant 1.82 and 1.91 in *Cedrus deodara* and *Cupressus torulosa*, respectively. In root trainers the morphological characters *viz*, plant survival (80.00 and 79.15 per cent), plant height(13.16 and 17.23 cm), collar diameter (2.25 and 2.47mm), shoot to root ratio (2.82 and 3.36), plant dry weight g/plant of 1.76 and 1.77 were maximum in *Cedrus deodara* and *Cupressus torulosa* respectively, in 300 cc root trainer size.

Keywords : Root Trainers, *Cedrus deodara, Cupressus torulosa, Pisolinthus tinctorius, Azotobacter*, potting media.

INTRODUCTION

Cedrus deodara is a species of Cedar native to Western Himalayas in Eastern Afghanistan, Northern Pakistan, North central India (H.P., Kashmir and Uttarakhand), South-Western most Tibet and Western Nepal, growing at 1500-3200 m altitude. It is a large evergreen coniferous tree of 40-50 m tall, with a trunk up to 3m diameter. It has conic crown with level branches and drooping branchlets. The deodar tree is the national tree of Pakistan. Among Hindus it is worshiped as a divine tree, particularly in the village of Kashmir and Himachal Pradesh, as the name deodar suggests. The first half of the word deva means the words divine, deity, dues, and Zeus and the second part connotes durum, druid, tree and true. The maximum area under deodar forest reported in Jammu and Kashmir is 113000 ha and is one of the most important and valuable timber of northern India. Seasoned heartwood of deodar is very durable and it is believed that deodar timber in certain buildings in Kashmir are 600 - 800 years old (Luna 1989), even the famous houseboats in Kashmir are made of deodar timber.

Cupressus torulosa commonly known as Himalayan cypress is found from Eastern Asia, Western China to Himalayas ranging from 1800 -3300 meters above mean sea level. It is an evergreen tree growing to 40 m height and in leaf all year. The scented flowers are monoecious and are pollinated by wind. It grows well in drier areas, especially on limestone.

Propagation of tree species through seed is most economical and practical but the problem in conifer seed is that they have an inherent problem of slow and poor germination both in field and laboratory conditions (Shopmeyer, 1974). The primary requirement for growing quality seedlings in the nursery in suitable medium. A good potting mixture is characterised with light weight, friability, easy blend ability, good water holding capacity and good drainage. (Chakravarti *et al.*, 1998). Different form of various manures, improves the physical properties *viz.*; porosity, cation exchange capacity (CEC), water holding capacity and of mycorrhizal technology for reforestation programme has become obligatory mainly due to the beneficial effects of mycorrhizae on growth and survival of plants (Kropp and Langlois 1990). It has been shown that forestry nursery plants exhibit complete dependence on mycorrhizae and do not get established in their absence. This has been attributed to the capability of mycorrhizal fungi to enhance the absorption of essential elements like N, P, K and relatively immobile elements Cu and Zn beyond nutrient depletion zone (Zargar *et al.*, 2004).

At present, the seedlings are being raised in the perforated polybags because of their low cost, simple working and convenient handling. The disadvantages of polybags are numerous and explained in great detail by many workers (Josiah and Jones, 1992; Khedkar and Subramaniam, 1997). Looking at the disadvantages of polybags, Josiah and Jones (1992) have favoured root trainer technology for raising plants in forest nurseries. Root trainers can deal with the problems of

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root coiling and distortion (Gera *et al.*, 1996). The plants raised in root trainers are capable of surviving in prolonged drought periods after planting because of complete root development in nursery. In addition to this it has several other advantages like easy handling and transportation, less space requirement, requirement of less quantity of potting material, easy root pruning etc. which ultimately saves cost and labour and makes the nursery management economical.

Keeping in view the significance of organic manures and mycorrhizae in the potting media and the importance of root trainer technology, the present investigation was carried out with the objective to devise an appropriate medium and root trainer for production of quality seedling of *Cedrus deodara* and *Cupressus torulosa*.

MATERIAL AND METHODS

The investigation was conducted in Forest nursery of Faculty of Forestry SKUAST - K, Shalimar during 2007-2008. For the experiment eight different potting media and two different root trainer sizes were used.

1. Potting medium

M_1 = Forest soil only.
M_2 = Forest Soil : Sand (1:1)
M_3 = Forest soil : Sand : FYM in the ratio of (1:1:1)
M_4 = Forest Soil : Sand : Mushroom Compost (1:1:1)
M_5 = Forest Soil : Sand : Dalweed (1:1:1)
M_6 = Forest Soil : Sand : *Pisolinthus tinctorius.*
M_7 = Forest Soil : Sand : *Coenococcum geophillum*
M_8 = Forest Soil : Sand : *Azotobacter.*

The well decomposed farm yard manure (FYM), dalweed and mushroom compost, consisting of already decomposed material left after growing mushrooms, were used for the experiment. Sand and forest soil, collected under the canopy of Deodar and Cupressus trees were sieved through normal mesh sieves to remove undecomposed leaves, stones, twigs and other foreign matter. All the materials were collected locally. The potting mixtures were thoroughly mixed in a desired proportion before filling pots. Microbial inoculants *viz, Pisolinthus tinctorius, Coenococcum geophillum* and *Azotobacter* were isolated from forest soil and were applied @ 50 ml per pot (10^6 CFU/ml) at the time of sowing. Seeds of both tree species *viz.*, Deodar and Cupressus were collected in November 2006 and were sown in perforated polybags of 5" x 7" in February 2007. The polybags were filled upto 85 % of their capacity. Sprouted seedlings were properly watered with help of sprinkler as and when required. Observation on morphological characters *viz.*, plant survival, seedling height, collar diameter, shoot root ratio, and plant dry weight were recorded after one complete growing season. The experiment was laid out in completely randomised design with four replications. In the second experiment, root trainers were filled with best potting medium obtained on the basis of the result of first experiment. Thereafter, root trainers were kept on raised platform made of wired mess to allow self air pruning of roots and encourage fibrous root system in root trainers. Seeds of both tree species were sown. Different morphological parameters of seedlings *viz.*, plant survival, plant height, collar diameter, shoot to root ratio and plant dry weight were recorded after one complete growing season.

RESULTS AND DISCUSSION

Perusal of the data in Table 1 shows mean values on various morphological parameters of *Cedrus deodara* seedlings raised under different combinations of potting mixtures. There were significant differences amongst the treatments with respect to their effect on plant survival, plant height, shoot to root ratio and total biomass. The maximum plant survival (75.05%) was observed in M_6 which consisted of Forest soil: Sand: *Pisolinthus tinctorus*. Similarly maximum plant height 14.60 cm, Collar diameter 1.96 mm and total biomass 1.82 was also recorded in M_6. Similarly the analysis of the data in Table 2 revealed that the seedlings of *Cupressus torulosa* raised under different combinations of potting mixtures also exhibited significant differences amongst the treatments with respect to their plant survival, plant height, shoot to root ratio and total biomass. The maximum plant survival 78.40% was also observed in M_6 which consisted of Forest soil: Sand: *Pisolinthus tinctorus* and maximum plant height 17.46 cm, collar diameter 1.99 mm , total biomass 1.91 , shoot to root ratio 3.92 was also recorded in M_6. The better growth of seedlings in potting mixtures of forest soil: Sand: *Pisolinthus tinctorus* may be due to mobilization of macro and micro nutrients and production of growth promoting substances by the ectomycorrhizae Zargar *et al.*, 2004 also reported similar findings.

The M_6 medium used in root trainers for raising the seedlings of Deodar, Cupressus and the results thus obtained are depicted in Table 3 and 4 respectively. The results shows that morphological parameters were better in case of 300 cc size of root trainers. The maximum plant survival 80.00, plant height 13.16, collar diameter 2.25 and shoot to root ratio 2.82 were recorded in Deodar raised in 300cc containers, while as it was 79.15 % plant height 17.23 cm, collar diameter 2.47 mm, Shoot to root ratio 3.36, plant dry weight (1.72) in case of seedlings of *Cupressus torulosa* raised in same size of root trainers (300cc). Sutherland and Day (1998) have also reported increase in seedling growth with increase in volume of container. Misra and Jaiswal (1993) reported maximum survival, height, Collar diameter, in case of seedling of Silver Oak, raised in biggest size of polybags.

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Table 1 : Effect of various organic manures and microbial inoculants on morphological parameters of *Cedrus deodara* seedlings.

Potting medium	Plant survival (%)	Plant height (cm)	Collar diameter (mm)	Shoot: root ratio	Plant dry weight (g plant^{-1})
M_1	53.42	9.12	1.52	2.39	1.08
M_2	63.15	10.43	1.55	2.69	1.19
M_3	66.61	11.00	1.13	3.00	1.30
M_4	68.31	11.22	1.60	3.47	1.41
M_5	70.56	11.58	1.76	3.53	1.51
M_6	75.05	14.60	1.96	3.82	1.82
M_7	70.10	11.09	1.70	3.49	1.51
M_8	72.47	12.17	1.01	3.40	1.50
CD(0.05)	0.948	0.594	0.04	0.057	0.023

Table 2 : Effect of various organic manures and microbial inoculants on morphological parameters of *Cupressus torulosa* seedlings.

Potting medium	Plant survival (%)	Plant height (cm)	Collar diameter (mm)	Shoot: root ratio	Plant dry weight (g plant^{-1})
M_1	56.33	10.75	1.59	2.51	1.15
M_2	66.23	12.06	1.76	2.85	1.34
M_3	71.23	12.18	1.15	3.17	1.38
M_4	72.87	12.43	1.82	3.60	1.59
M_5	73.01	12.69	1.16	3.44	1.15
M_6	78.40	17.46	1.99	3.92	1.91
M_7	72.85	12.80	1.94	3.74	1.73
M_8	73.49	12.37	1.09	3.44	1.58
CD(0.05)	0.954	0.676	0.443	0.076	0.028

Table 3 : Effect of root trainer size on seedling growth of *Cedrus deodara*.

Size of root trainers (cc)	Plant survival (%)	Plant height (cm)	Collar diameter (mm)	Shoot: root ratio	Plant dry weight (g plant^{-1})
150 cc	70.46	11.45	1.072	2.67	1.30
300 cc	80.00	13.16	2.255	2.82	1.76
CD (0.05)	1.46	1.153	0.209	0.100	0.042

Table 4 : Effect of root trainer size on seedling growth of *Cupressus torulosa*.

Size of root trainers (cc)	Plant survival (%)	Plant height (cm)	Collar diameter (mm)	Shoot: root ratio	Plant dry weight (g plant^{-1})
150 cc	73.27	11.97	1.91	2.83	1.37
300 cc	79.15	17.23	2.47	3.36	1.77
CD (0.05)	0.299	1.952	0.199	0.033	0.079

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Studies of Leaf Surface Mycoflora of *Barleria prionitis* L.

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During present studies leaf surface mycoflora of *Barleria prionitis* was observed fortnightly with the help of gravity pertiplate method from July 2003 to June 2004. 38 fungal species (568 colonies) were observed from the leaf surface mycoflora during July 2003 to June 2004. Maximum 30 fungal species were observed during the winter season, 25 in rainy season and minimum 20 in summer season. Among the mycoflora, 38 fungal species, 3 from Zygomycotina, 2 from Ascomycotina and 33 from Anamorphic fungi were recorded.

Keywords : *Barleria prionitis,* Mycoflora.

INTRODUCTION

Air is one of the most important ingredient of environment like soil and water. Plants, animals and human beings are continuously facing the attack of microorganisms, specially bacteria and fungi attack the biotic flora and deteriorate food and damage crops.

Barleria prionitis is medicinal plant belong to family Acanthaceae is selected for present investigation which were cultivated in Botanical Garden of Govt. Science College Raipur. During present studies of leaf surface mycoflora of above plant were observed fortnightly with the help of gravity pertriplate methods from July 2003 to June 2004. Aspect related to leaf surface mycoflora associated with several plant have been studied by Sharma (1973a), Tiwari (1977), Tilak and Kulkarni (1978), Tiwari and Sahu (1989 a,b), Aher *et al.* (2002), Tilak and Gardekar (2005) and Uddin (2005).

MATERIAL AND METHODS

For the survey of leaf surface mycoflora, leaves were collected at random from any plant, each time. Sample leaves were collected in sterilized polythene bags. Then brought into the laboratory for the isolation of the leaf surface mycoflora. In the laboratory the collected leaves were placed in conical flask containing 75 ml of sterilized distilled water. The flask was hand shaken for 30 minutes to make a homogenous suspension of fungal species. 1 ml of this suspension poured into the petriplate containing Potato Dextrose Agar (PDA) medium. 5 petriplates were used at a time in each experiment. Then the plates were incubated at 26 ± 1°C in the incubation chamber for 6-7 days. After the incubation, the fungal colonies were counted then identified and maintained the pure culture.

For the seasonal study of leaf surface mycoflora, meteorological data (temperature, relative humidity and rainfall) obtain from the meteorological department of Indra Gandhi Krishi Vishwavidyalaya, Raipur (C.G.)

RESULTS AND DISCUSSION

During this work 568 fungal colonies, representing 38 species and 17 genera were isolated (Table.1). Among 38 fungal species, 3 from Zygomycotina, 2 from Ascomycotina and 33 from Anamorphic fungi were observed. During investigation, 30 fungal species were observed in winter season, 25 in rainy season and 20 in summer season (Fig. 1).

30 fungal species (225 colonies) were observed in winter season. Out of 30 fungal species 3,1,26 from Zygomycotina, Ascomycotina, Anamorphic fungi (Fig. 2).

In rainy season, (200) fungal colonies of 25 fungal species were recorded. Among 25 fungal species, 2,1,22 from Zygomycotina, Ascomycotina, Anamorphic fungi (Fig. 3).

While in summer season 20 fungal species (143 colonies) 1,2,17 from Zygomycotina, Ascomycotina, Anamorphic fungi were recorded (Fig. 4).

Leaf surface mycoflora together with meteorological factors reflect that the fungal population present on the leaf surface effect by temperature, relative humidity and rainfall.

During the present study, maximum fungal species (30) were recorded in winter season, (max temp 28.46 °C min 13.30 °C RH-87.25 and Avg. Rainfall 190mm) due to favorable temperature, relative humidity and rainfall, similar result were also observed by Gegory (1971), Mishra and Shrivastava (1971a). Minimum fungal species (20) have recorded in summer season (max temp 38.4 °C min 28 °C RH-55.6 and Avg. rainfall 10- 15mm) due to un favorable condition, also observed by Navneet and Mehrotra (1987), Tiwari and Sahu (1991), Tiwari *et al.* (1993) while moderate fungal species (25)

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Table 1 : Showing the leaf surface mycoflora of *Barleria prionitis* Linn.

S. No.	Name of Fungi	Rainy Season					Winter Season					Summer Season					Total Coloy
		Jul.	Aug.	Sept.	Oct.	Total	Nov.	Dec.	Jan.	Feb.	Total	Mar.	Apr.	May	June	Total	
	ZYGOMYCOTINA																
1.	*Rhizopus rhizopodiformis*	1				1			2		2						3
2.	*Choanephora cucurbitarum*	4				4	2				2						6
3.	*Mucor* sp.									2	2			1		1	3
	ASCOMYCOTINA																
1.	*Thielavia boothi*													1		1	1
2.	*Chaetomium aureum*	2				2			2		2				2	2	5
	ANAMORPHIC FUNGI																
1.	*Alternaria radicina*	4			5	9	2		6	7	15						24
2.	*Alternaria citri*	6		2	5	13	8	6			14			2	4	6	33
3.	*Alternaria alternata*	4	4			8	5		5	3	13	10	2	1	4	17	38
4.	*Alternaria chlamydospora*							1	3		4						4
5.	*Aspergillus niger*	6	4	3	5	18	2	3	5	8	18	4	6	1	9	20	56
6.	*Aspergillus fumigatus*	2				2						9	7	3	3	22	24
7.	*Aspergillus ustus*	3				3								3	2	5	8
8.	*Aspergillus stellatus*	5				5											5
9.	*Aspergillus flavus*	3	6	3	1	13	2	1			3	12	3	2	2	19	35
10.	*Aspergillus nidulans* var. acristatus														2	2	2
11.	*Aspergillus awamori*	1		2		3	6	3			9						12
12.	*Aspergillus nidulans*	2				2							2	1		3	5
13.	*Cladosporium oxysoprum*	5			8	13	6	5	6	4	21	6	4			10	44
14.	*Cladosporium cladosporioids*		5		5	10	4	8	9		21	5				5	36
15.	*Curvularia lunata*	4	7	5		16	12	7			19			2	6	8	43
16.	*Curvularia clavata*	2	8	4	6	20	5				5			2		2	27
17.	*Curvularia eragrostidis*								3	2	5	4	2			6	11
18.	*Curvularia oryzae*									1	1	1				1	2
19.	*Curvularia senegalensis*							1			1						1
20.	*Colletotrichum dematium*		5	6		11				3	3						14
21.	*Drechslera australiensis*								2		2						2
22.	*Drechslera halodes*													2	3	5	5
23.	*Drechslera hawaiiens*												1			1	1
24.	*Fusarium moniliforme*						4	3	2		9						9
25.	*Fusarium oxysporum*	8	8	7	6	29	2	3			5						34
26.	*Nigrospora oryzae*	2				2	2	1			3						5
27.	*Nigrospora sphaerica*				8	8	7	8	6	6	27						35
28.	*Penicillium notatum*								4		4						4
29.	*Penicillium rugulosum*	2				2	1				1	1	4			5	8
30.	*Pithomyces graminicola*	2				2	3				3						5
31.	*Phoma* sp.							3	2	2	7						7
32.	*Mycelia sterilia* black		1			1			1	1	2						3
33.	*Mycelia sterilia* white	1		2		3		2			2		3			3	8
	TOTAL	**62**	**48**	**34**	**49**	**163**	**71**	**55**	**54**	**37**	**171**	**52**	**34**	**19**	**35**	**117**	**451**
	TOTAL COLONIES	**69**	**48**	**34**	**49**	**200**	**73**	**55**	**58**	**39**	**225**	**52**	**34**	**21**	**36**	**143**	**568**

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Fig. 1 : **Showing fungal species of leaf surface mycoflora in different season of *Barleria prionitis*.**

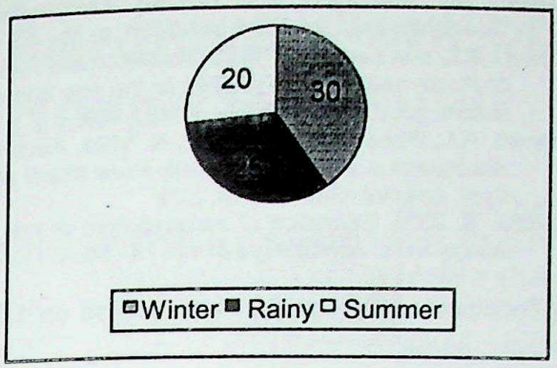


Fig. 2 : **Showing the total no. of fungal species and different class of leaf surface mycoflora in winter season of *Barleria prionitis*.**

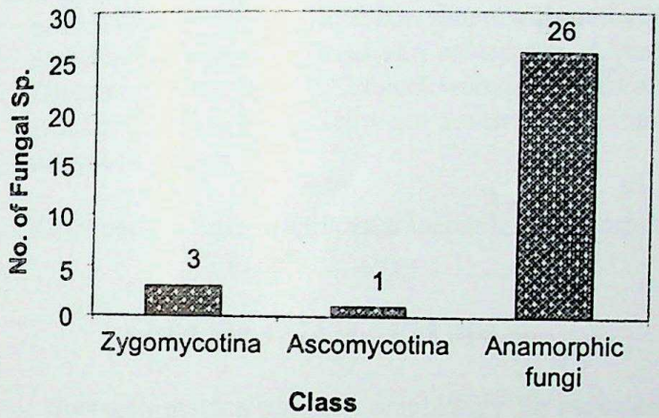
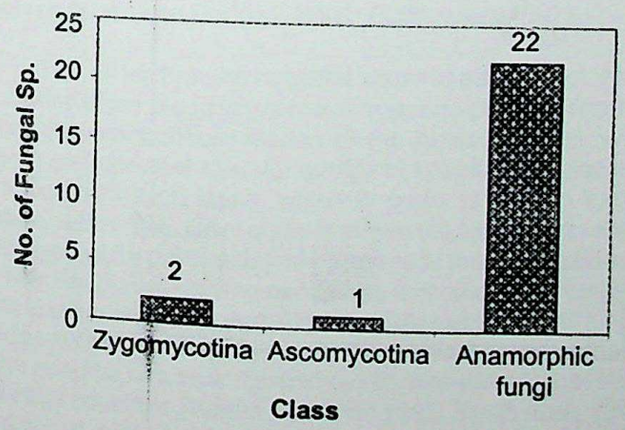


Fig. 3 : **Showing the total no. of fungal species and different class of leaf surface mycoflora in rainy season of *Barleria prionitis*.**



were recorded in rainy season, (max temp 32.86 °C min 24 °C, RH-92.5 and Avg. Rainfall) on leaf surface (Fig. 5).

Fig. 4 : **Showing the total no. of fungal species and different class of leaf surface mycoflora in summer season of *Barleria prionitis*.**

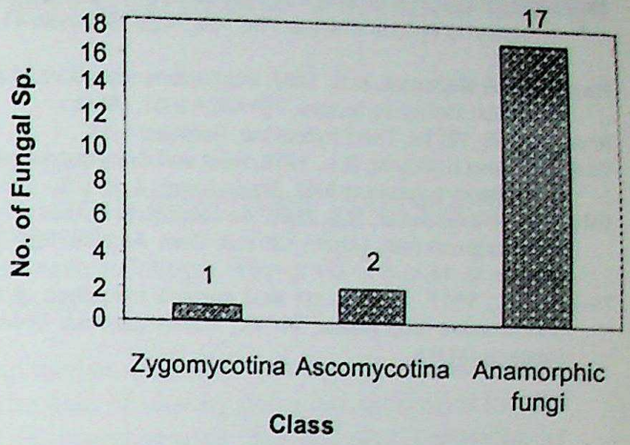
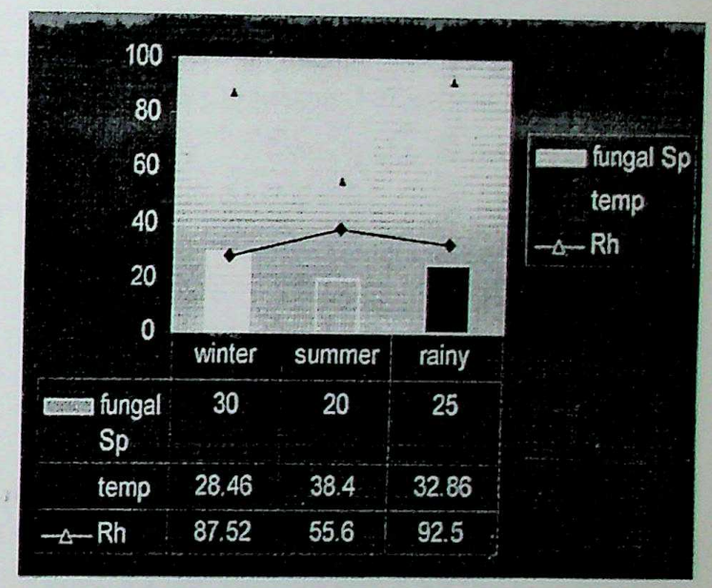


Fig. 5 : **Showing seasonal wise distribution of fungal species.**



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Seasonal Variation of Indoor Aeromycoflora of Dr. Bhim Rao Ambedkar Hospital, Raipur

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Aeromycological studies of indoor environments of the hospital was studied in relation to environmental factors *i.e.*, temperature, rainfall and relative humidity with the help of Gravity petriplate technique for the period of one year from July 2005 to June 2006. A total of 96 fungal species (985 colonies) belonging to 36 genera were recorded during the investigation. Seasonal variations in the incidence of fungal species and colonies were observed and correlated with meteorological conditions. *Alternaria alternata, A. citri, Aspergillus aculeatus, A. flavus, A. flavus* var. *columnaris, A. fumigatus, A. niger, A. versicolor, Cladosporium cladosporioides, Curvularia eragrostidis* and *C. lunata* were present throughout the season. It has been also observed that maximum numbers of fungal species were assessed in winter season while minimum numbers of fungal species in summer season.

Keywords : Aeromycoflora, Hospital, Environmental factors.

INTRODUCTION

During the last few decades, aerobiology has assumed in diagnosis of fungal allergic disorders. Various systematic studies were conducted on the presence of fungal spores in the atmosphere and their effect on human health. Fungal spores are among the most commonly encountered airborne particles. Some fungi and their spores have a negative effect on human health and are frequent cause of allergies of fungal diseases (Bush and Portnoy, 2001; Dutkiewicz, 1997).

The air we breathe contains spore of many fungi. Air is the carrier of the bioparticles move from one place to another place. Therefore dissemination of the fungal spores, air is playing an important role. Air quality of hospital has become an important health issue, which is partly related to fungal contamination. The aim of study is to give the information about which type of fungal species are present in the atmosphere of the hospital during varying conditions and also importance in order to understand the deposition and dissemination of fungal species inside the hospital. Several works in the environments of the hospital has been carreid out in different parts of India and other countries (Verma and Chile 1992; Singh *et al.*, 1994; Nasim and Wahid, 1998; Kulshrestha and Chauhan, 2001; Sahney and Purwar, 2002; Agashe *et al.*, 2002; Hardin *et al.*, 2003; Martins-Diniz *et al.*, 2005; Perdelli *et al.*, 2006; Kalkar and Tattle.2007).

STUDY AREA

The present investigation was carried out at Dr. Bhim Rao Ambedkar Hospital, Raipur. It is situated near the Central Jail, Railway station and crowd area of the Raipur city. Raipur city is situated between 22° - 33' N to 21° - 14' North latitude and 82°- 38' East longitude about 298.60 meters above the sea level. It occupies the South Eastern part of the upper Mahanadi river valley and the bordering hills in the South and the East thus the district is divided into two major physical division: the Chhattisgarh plains and the hilly areas. Raipur has a tropical wet and dry climate, temperature remain moderate for most of the year, apart from the summer from March to June, which can be extremely hot. The city receives about 1300 mm of rain, mostly in the monsoon season from late June to early October. Winter last from November to February and are although lows can fall to10°C.

MATERIAL AND METHODS

The study was conducted for the periods of one year from July 2005 to June 2006. Air sampling was carried out by using sterilized Petriplates containing PDA (Potato-Dextrose-Agar) medium. Five plates were exposed for indoor aeromycoflora of pre-surgical ward, post-surgical ward, female, male surgical ward and dressing room of the hospital for 5-10 minutes at fortnightly interval. The exposed petriplates were brought into the laboratory and incubated at 26 ± 1°C for

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incubation period. After incubation period fungal colonies were counted, isolated and identified with the help of available literature and finally determined/confirmed by the authentic authority.

RESULTS

Seasonal variations were noted in prevalence of total fungal colonies and their species. Seasonal variation of aeromycoflora depends on environmental factors such as temperature, relative humidity and rainfall.

In the present study, 96 fungal species (985 colonies) belonging to 36 genera have been recorded. Out of 96 fungal species, 08 fungal species (21 colonies) having 06 genera belonging to group Zygomycotina. 05 species (13 colonies), 04 genera belonging to Ascomycotina. 79 species (903 colonies), 25 genera to Anamorphic fungi and 04 species (48 colonies), 01 genera belonging to mycelia sterilia (Fig-1). Maximum 67 fungal species (406 colonies) were observed during winter season, moderate 54 fungal species (332 colonies) in rainy season and minimum 39 fungal species (247 colonies) were observed in summer season. (Table-1, Fig-2).

In rainy season, a total of 54 fungal species (332 fungal colonies) were observed. Out of total 54 fungal species, 05 species (14 colonies) from Zygomycotina, 01 species (04 colonies) from Ascomycotina, 46 species (295 colonies) from Anamorphic fungi and 02 species (19 colonies) from Mycelia sterilia were observed (Table 1).

In winter season, a total of 67 fungal species (406 colonies) were observed. Among them, 03 species (05 colonies) from Zygomycotina, 02 species (04 colonies) from Ascomycotina, 58 species (379 colonies) from Anamorphic fungi and 04 species (18 colonies) from Mycelia sterilia were observed (Table-1).

During summer season, a total of 39 fungal species (247 colonies) were observed. Out of these, 02 species (02 colonies) from Zygomycotina, 04 species (05 colonies) from Ascomycotina, 31 species (229 colonies) from Anamorphic fungi and 02 species (11 colonies) from Mycelia sterilia were observed during the summer season in indoor aeromycoflora. (Table 1)

During monthly variation of indoor aeromycoflora, maximum 42 fungal species were observed in December while minimum 16 fungal species in the month of May (Fig-3).

It was also observed that some fungi were present in all the seasons of indoor aeromycoflora *i.e., Alternaria alternata, A. citri, Aspergillus aculeatus, A. flavus, A. flavus* var. *columnaris, A. fumigatus, A. niger, A. versicolor, Cladosporium cladosporioides, Curvularia eragrostidis, C. lunata, C. pallescens* from Anamorphic fungi and Mycelia sterilia (Grey black).

Some fungal species were present more than one season but not in all seasons of indoor aeromycoflora such as *Absidia spinosa, Rhizopus nigricans* from Zygomycotina, *Emericella nidulans, Lewia infectoria* from Ascomycotina, *Alternaria cheiranthi, A. chlamydospora, A. radicina, A. pluriseptata, Aspergillus japonicus, A. ochraceus, A. parasiticus, A. rugulosus, A. speluneus, A. stellatus, A. terreus, A. ustus, Cladosporium sphaerospermum, Corynespora cassicola, Curvularia brachyspora, C. clavata, C. geniculata, Drechslera hawaiiensis, Fusarium* sp. III, *Humicola* sp., *Monodictys fluctuata, Nigrospora oryzae, Paecilomyces variotii, Penicillium funiculosum, P. multicolor, P. rugulosum, P. turbatum, Penicillium* sp., *Periconia atra, Phoma glomerata, Pithomyces chartarum, Scytalidium* state of *Hendersonula toruloidea* from Anamorphic fungi, Mycelia sterilia (Chlamydospora) and Mycelia sterilia (White).

Similarly certain fungi were restricted only in one season of indoor aeromycoflora such as, *Choanephora cucurbitarum, Cunnighamella elegans, Mucor racemosus, Mucor* sp., *Rhizopus nodosus, Syncephalastrum racemosum* from Zygomycotina, *Chaetomium atrobrunneum, Chaetomium* sp., *Thielavia terricola* from Ascomycotina, *Alternaria brassicicola, A. raphani, Alternaria* sp., *Arthrinium phaeospermum, A. saccharicola, Ascotricha chartarum, Aspergillus carneus, A. chevalieri* var. *intermedius, A. flavipes, A. nidulans* var. *aeristatus, A. petrikii, Aureobasidium pullulans, Cladosporium herbarum, Corynespora* sp., *Curvularia ovoidea, C. trifolii, Drechslera* state of *Trichometasphaeria holmii, Drechslera* state of *Trichometasphaeria pedicellata, Epicoccum nigrum, Fusarium* sp.IV, *Humicola fuscoatra, Monilia* sp., *Nigrospora sphaerica, Penicillium aurantiocandidum, P. frequentans, P. humuli, P. purpurogenum, Periconia* sp., *Pestalotiopsis glandicola, Phoma fimeti, Phoma* sp., *Sclerotium* sp., *Trichoderma* sp.I, *Trichoderma* sp.II, *Trichothecium roseum* from Anamorphic fungi and Mycelia sterilia (Pink).

DISCUSSION

Seasonal variations were noted in prevalence of total fungal colonies and species. Meteorological condition plays an important role on the incidence of fungal species. Maximum number of fungal species *i.e.*, 67 (406 colonies) was observed during winter season in indoor environments of the hospital. During the investigation periods, monthly variations of aeromycoflora were also recorded. Maximum incidences of fungal species (42) were recorded during the month of December, coincides with the optimum temperature (27.3°C, R.H. 89.2%) favorable for the fungal growth. With the increase in temperature in April (39.2°C, R.H. 73.7%) concentration of fungal species start decreasing, reaching its minimum (16) in May which was maximum temperature (40.2°C, R.H. 56.6%) and dry month of Raipur. During rainy season high relative humidity (91.8%) and minimum temperature (30.7°C), favour fungal sporulation, but due to rains clear the atmosphere resulting a remarkable decrease in the fungal species as compared to winter season. In rainy season, maximum incidence of fungal species (29) are observed in the month of August where temperature and humidity (29.9°C, R.H. 92.2%) slightly favorable for the fungal growth, than again decrease in fungal species in July, October and September respectively, due to slightly increase in the temperature ranging between

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Table 1.

S. No.	NAME OF FUNGI	RAINY SEASON	WINTER SEASON	SUMMER SEASON	GRAND TOTAL OF FUNGAL COLONIES
	ZYGOMYCOTINA				
01.	*Absidia spinosa*	02	01	-	03
02.	*Choanephora cucurbitarum*	02	-	-	02
03.	*Cunninghamella elegans*	01	-	-	01
04.	*Mucor racemosus*	-	03	-	03
05.	*Mucor* sp.	-	-	01	01
06.	*Rhizopus nigricans*	06	01	-	07
07.	*R. nodosus*	-	-	01	01
08.	*Syncephalastrum racemosum*	03	-	-	03
Total no. of fungal colonies		14	05	02	21
Total no. of fungal species		05	03	02	08
	ASCOMYCOTINA				
09.	*Chaetomium atrobrunneum*	-	-	02	02
10.	*Chaetomium* sp.	-	-	01	01
11.	*Emericella nidulans*	04	-	01	05
12.	*Lewia infectoria*	-	02	01	03
13.	*Thielavia terricola*	-	02	-	02
Total no. of fungal colonies		04	04	05	13
Total no. of fungal species		01	02	04	07
	ANAMORPHIC FUNGI				
14.	*Alternaria alternata*	08	19	28	55
15.	*A. brassicicola*	-	01	-	01
16.	*A. cheiranthi*	03	02	-	05
17.	*A. chlamydospora*	-	02	02	04
18.	*A. citri*	07	08	17	32
19.	*A. pluriseptata*	02	02	-	04
20.	*A. radicina*	-	08	06	14
21.	*A. raphani*	-	10	-	10
22.	*Alternaria* sp.	-	02	-	02
23.	*Arthrinium phaeospermum*	04	-	-	04
24.	*A. saccharicola*	-	02	-	02
25.	*Ascotricha chartarum*	-	-	05	05
26.	*Aspergillus aculeatus*	01	03	05	09
27.	*A. carneus*	-	01	-	01
28.	*A. chevalieri* var. *intermedius*	06	-	-	06
29.	*A. flavipes*	-	02	-	02
30.	*A. flavus*	01	11	10	22
31.	*A. flavus* var. *columnaris*	05	03	02	10
32.	*A. fumigatus*	02	02	04	08
33.	*A. japonicus*	04	-	04	08
34.	*A. nidulans* var. *aeristatus*	-	02	-	02
35.	*A. niger*	12	08	12	32

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36.	*A. ochraceus*	06	-	04	10
37.	*A. parasiticus*	-	02	03	05
38.	*A. petrikii*	-	-	01	01
39.	*A. rugulosus*	01	02	-	03
40.	*A. speluneus*	03	02	-	05
41.	*A. stellatus*	02	-	01	03
42.	*A. terreus*	04	03	-	07
43.	*A. ustus*	02	01	-	03
44.	*A. versicolor*	73	16	17	106
45.	*Aureobasidium pullulans*	-	01	-	01
46.	*Cladosporium cladosporioides*	49	132	44	225
47.	*C. herbarum*	-	03	-	03
48.	*C. sphaerospermum*	14	22	-	36
49.	*Corynespora cassicola*	02	02	-	04
50.	*Corynespora* sp.	-	01	-	01
51.	*Curvularia brachyspora*	02	05	-	07
52.	*C. clavata*	03	04	-	07
53.	*C. eragrostidis*	01	07	01	09
54.	*C. geniculata*	-	04	04	08
55.	*C. lunata*	11	08	10	29
56.	*C. ovoidea*	-	02	-	02
57.	*C. pallescens*	05	05	11	21
58.	*C. tifolii*	-	03	-	03
59.	*Drechslera hawaiiensis*	04	-	05	09
60.	*Drechslera* state of *Trichometasphaeria holmii*	-	01	-	01
61.	*Drechslera* state of *Trichometashaeria pedicellata*	02	-	-	02
62.	*Epicoccum nigrum*	02	-	-	02
63.	*Fusarium* sp. III	04	02	-	06
64.	*Fusarium* sp. IV	-	02	-	02
65.	*Humicola fuscoatra*	-	02	-	02
66.	*Humicola* sp.	02	-	03	05
67.	*Monilia* sp.	-	06	-	06
68.	*Monodictys fluctuata*	01	02	-	03
69.	*Nigrospora oryzae*	05	07	-	12
70.	*N. sphaerica*	-	05	-	05
71.	*Paecilomyces variotii*	04	-	05	09
72.	*Penicillium aurantiocandidum*	03	-	-	03
73.	*P. frequentans*	-	10	-	10
74.	*P. funiculosum*	04	01	-	05
75.	*P. humuli*	-	02	-	02
76.	*P. multicolor*	-	04	04	08
77.	*P. purpurogenum*	13	-	-	13
78.	*P. rugulosum*	-	06	03	09
79.	*P. turbatum*	-	01	02	03
80.	*Penicillium* sp.	-	04	03	07

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81.	*Periconia atra*	01	01	-	02
82.	*Periconia* sp.	-	03	-	03
83.	*Pestalotiopsis glandicola*	02	-	-	02
84.	*Phoma fimeti*	05	-	-	05
85.	*P. glomerata*	-	03	06	09
86.	*Phoma* sp.	02	-	-	02
87.	*Pithomyces chartarum*	01	02	-	03
88.	*Sclerotium* sp.	03	-	-	03
89.	*Scytalidium* state of *Hendersonula toruloidea*	02	-	02	04
90.	*Trichoderma* sp. I	-	-	05	05
91.	*Trichoderma* sp. II	02	-	-	02
92.	*Trichothecium roseum*	-	02	-	02
Total no. of fungal colonies		295	379	229	903
Total no. of fungal species		46	58	31	79
	MYCELIA STERILIA				
93.	Mycelia sterilia (Chlamydospora)	-	02	04	06
94.	Mycelia sterilia (White)	10	07	-	17
95.	Mycelia sterilia (Pink)	-	03	-	03
96.	Mycelia sterilia (Grey Black)	09	06	07	22
Total no. of fungal colonies		19	18	11	48
Total no. of fungal species		02	04	02	04
GRAND TOTAL OF FUNGAL COLONIES		332	406	247	985
GRAND TOTAL OF FUNGAL SPECIES		54	67	39	96

30.6°C to 31.4°C. (Fig. 4) These findings are also supported by those of Agarwal and Shivpuri (1974) and Kulshrestha and Chauhan (2001) who have supported that too hot condition are unfavourable for the sporulation of fungal spores. Verma and Pandey (1992) recorded highest percentage incidence of fungal spores in January followed by November and least in August in a hospital ward at Jabalpur. Atluri and Padmini (2002) reported highest incidence of fungal spores in July followed by August, September, June, March, January, December, February, November, October, April and May from main library of the Andhra University at Visakhapatanam. Seshadri (2003) have also reported that the minimum number of fungal spores in the month of May.

Maximum number of fungal species was recorded during winter season, moderate to the rainy season and minimum in the summer season is in accordance with the results of Singh *et al.* (1994), Tiwari *et al.* (1995, 2005), Jadhav, 1996; Kulshrestha and Chauhan (2001), Majumdar and Braui (2005), Kunjam (2007) and Mahajan and Cholke (2007).

CONCLUSION

The occurrence of different fungal species in the air of the hospital varied with varying seasons of the year, temperature, relative humidity, population and others. *Alternaria alternata, A. citri, Aspergillus aculeatus, A. flavus, A. flavus* var. *columnaris, A. fumigatus, A. niger, A. versicolor, Cladosporium cladosporioides, Curvularia eragrostidis* and *C. lunata* were present throughout the season and which can be harmful in nature for human beings in future. These results may be helpful to allerologists and clinician in treatment of fungal related diseases and allergy.

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Fig. 1 : Showing class wise distribution of indoor aeromycoflora

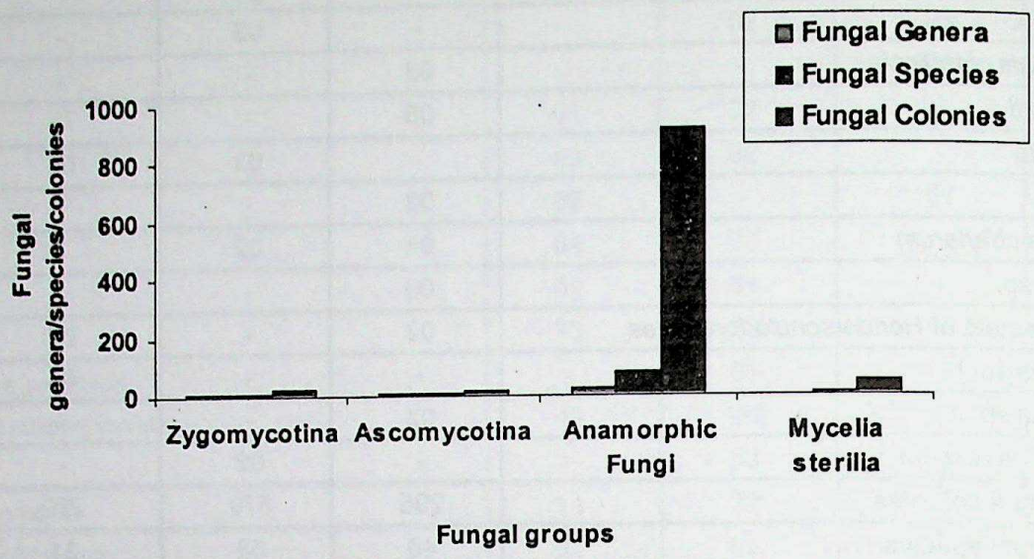

Fig. 2 : Showing season wise fungal species distribution of indoor aeromycoflora

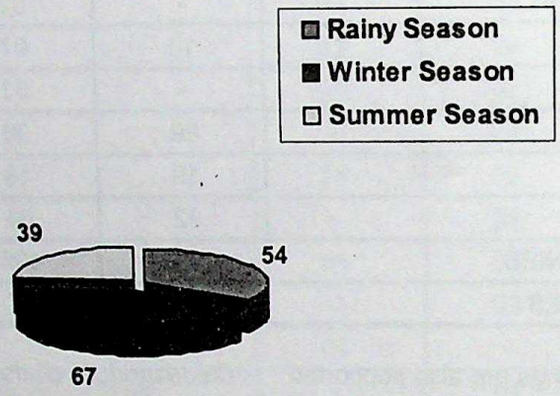

Fig. 3 : Showing month wise total no. of fungal species and fungal colonies

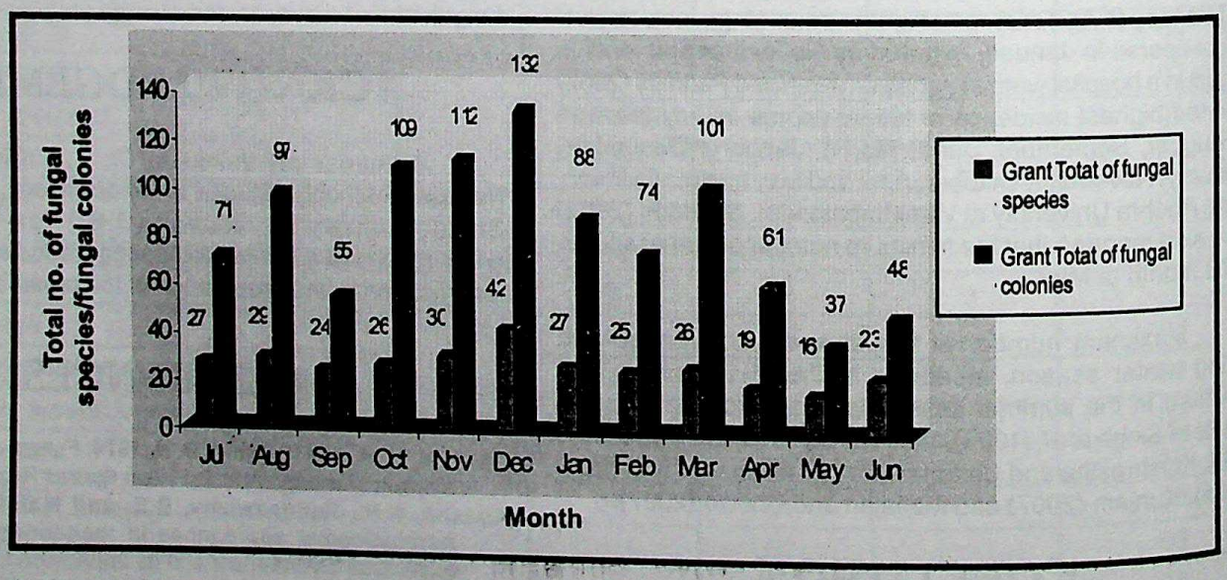

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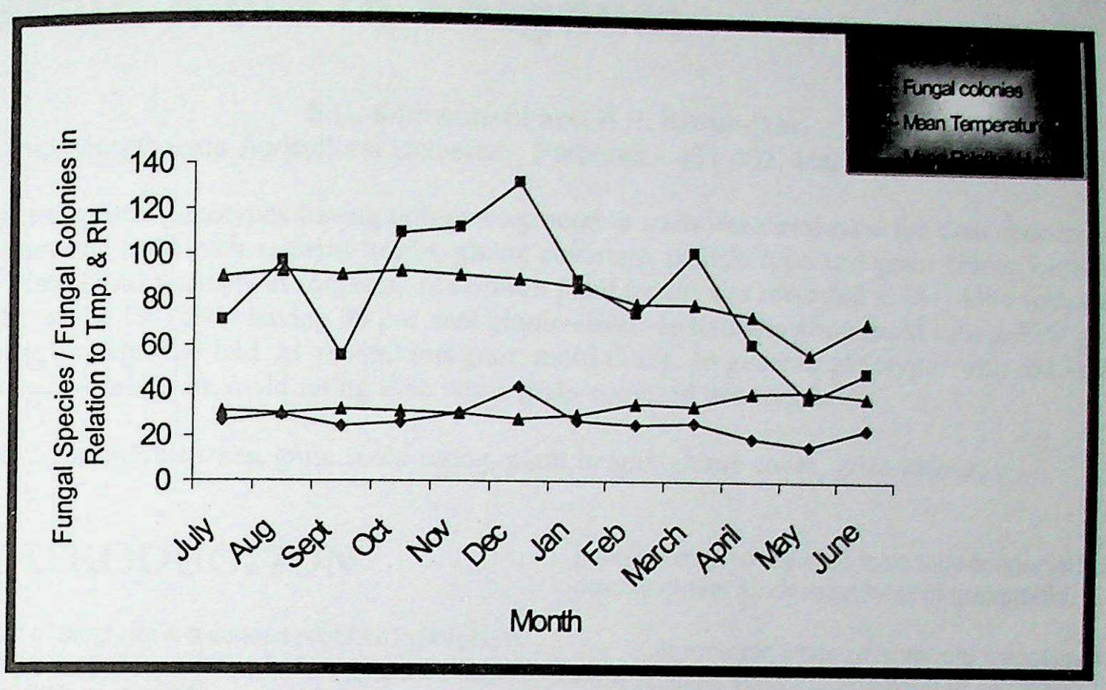
Fig. 4 : Showing compares the total no. of fungal species and fungal colonies with the environmental factors *i.e.* Temp. / Relative humidity during July 2005 - June 2006.



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Effect of Agronomic Traits on Intensity of Grain Mold in Sorghum

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Thirty eight sorghum genotypes having different agronomic traits were evaluated for their reaction to grain mold. Agronomic traits such as plant height, glume coverage, panicle type and grain colour were found to influence grain mold intensity in sorghum. Maximum plant height was recorded in IS–14384 with minimum grain mold rating. IS–14384 having 90 per cent glume coverage had less grain mold rating. Few genotypes having loose panicle type had in general less grain mold rating. In general, genotypes with red and brown grain colour had less grain mold rating than white grain coloured genotypes.

Keywords : Agronomic traits, grain mold rating, plant height, glume cover, grain colour.

INTRODUCTION

Grain mold of sorghum is a serious problem in sorghum grown during rainy season, especially when sorghum is caught in rains during different grain developmental stages. Management of grain mold is a difficult task as recommended sorghum cultivars do not possess appreciable resistance to mold. Grain mold has been reported to be favoured because of high humid conditions for longer time around developing grains (Siddiqui and Khan, 1973; Bhagwat and Datar, 1974; Seitz *et al.*, 1975; Williams and Rao, 1977; Koteshwara Rao, 1986; Indira *et al.*, 1991 and Somani, 1992). High humid conditions around developing grains is influenced because of agronomic traits such as crop canopy, height of the plant, panicle morphology etc. (Murthy, 1975, Gangadharan *et al.*, 1978, Glueck and Rooney, 1980 and Narayana and Prasad, 1980). Indira and Rana (1977) suggested that, longer glumes on the seed can be utilized as criteria in selecting for resistance to grain mold. In the light of this study, 38 sorghum genotypes having different agronomic traits were evaluated for their reaction to grain mold during the years 2002 and 2003.

MATERIAL AND METHODS

Thirty eight sorghum genotypes received from ICRISAT, Patancheru, Hyderabad were planted at sorghum Research Station, M.A.U., Parbhani during the year 2002 and 2003 for evaluation of their reaction against sorghum grain mold. Sorghum genotypes were planted in two rows of 4.5 m length. Genotypes were dibbled at 45 x 15 cm² distance. Recommended fertilizer doses were applied and three hoeings and three hand weedings were done for better growth of the crop. Thiodon 2 per cent and dimethoate 2.5 per cent insecticidal applications were carried out for management of shoot fly and aphids. Sprinkler irrigation was applied 4 hrs/day (2 hrs in morning and 2 hrs in evening) for 15 days during grain development stages to provide congenial humid condition around grains for development of grain mold.

Agronomic traits of sorghum genotypes under study were studied. Brief methodology of recording these traits is narrated below.

Plant height was measured at crop harvest from the base of the stem near ground level to the top of earhead at physiological maturity and crop maturity as per harvesting stages.

Panicle morphology of the sorghum genotypes under study was recorded into broad groups as loose, semi–compact and compact types, respectively.

Visible glume colours of the sorghum genotypes were recorded into broad groups as white, brown and red, respectively.

Observations on grain covering *i.e.,* amount of grain covered by the glume for all the genotypes under study were recorded. Mean of 25 glumes per genotype was considered as mean glume covering for same genotype.

Sorghum genotypes were categorized into mold broad types as having white, brown and red coloured grains.

Grain mold rating was done as field gain mold rating (FGMR) and threshed grain mold rating (TGMR) as per procedure given by Thakur *et al.*, (2003).

RESULTS AND DISCUSSION

Perusal of results depicted in Table 1 indicate that agronomic traits of sorghum genotypes such as plant height, panicle morphology, glume coverage, glume colour and grain colour had considerable variation between sorghum genotypes.

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Maximum plant height was recorded in IS–14384 during both the years of experimentation, which was significantly higher than rest of the genotypes. Plant height was minimum in Bulk–Y *i.e.*, 129.00 cm (Year 2002) and 114.00 cm (Year 2003) which was significantly less as compared to other genotypes during respective years. Rest of the genotypes had height from 139.33 cm to 207.00 cm during 2002 and 135.00 cm to 205.00 cm during 2003. Grain mold rating *i.e.*, FGMR and TGMR in IS–14384 was 2.75 and 2.50, in Bulk–Y 4.08 and 4.08 and in rest of the genotypes it varied from 3.16 to 3.91 and 3.08 to 4.08, respectively during 2002. In 2003, grain mold rating *i.e.*, FGMR and TGMR in resistant check IS–14384 was 1.36 and 1.00, in Bulk–Y 4.66 and 4.29 and in rest of the genotypes it varied from 1.58 to 4.33 and 1.16 to 3.95, respectively.

Glume coverage of sorghum genotypes ranged from 25 per cent (296–B, Bulk–Y, ICSB–383, ICSB–368, ICSB–362, ICSB–352, SGMR–24, SGMR–17, SGMR–12, SGMR–10) to 90 per cent (IS–14384). SGMR–3 was another genotype having higher glume coverage (75 per cent). Rest of the genotypes had 50 per cent glume coverage. Grain mold rating *i.e.*, FGMR and TGMR in these different genotypes having glume coverage upto 25 per cent ranged between 2.41 to 4.16 and 3.00 to 4.33, respectively. It ranged from 3.00 to 4.25 and 2.75 to 4.33, respectively in 50 per cent glume covering. The FGMR and TGMR ratings ranged from 3.25 and 3.50, respectively in SGMR-3 (75 per cent glume covering) and 2.75 and 2.50 in resistant check IS-14384 (90 per cent glume covering) during 2002. In 2003, grain mold rating *i.e.*, FGMR and TGMR in 25 per cent glume covering genotypes ranged between 1.58 to 4.66 and 1.11 to 4.29, respectively. It ranged between, 1.50 to 2.66 and 1.33 to 2.45, respectively in 50 per cent glume covering and from 2. 08 and 1.75, respectively in SGMR-3 (75 per cent glume covering). It was 1.36 and 1.00, respectively in IS-14384 having 90 per cent glume covering.

Panicle type in sorghum genotypes was either loose. semi-compact or compact. Genotypes Bulk–Y and IS–14384 had loose panicle type while 296–B, ICSB–383, ICSB–382 and ICSB-363 had compact type of panicle. Rest of the genotypes had semi–compact panicle type. Grain mold rating *i.e.*, FGMR and TGMR in these different panicle type genotypes ranged between 2.75 to 4.08 and 2.50 to 4.08, respectively in loose type, from 3.08 to 3.91 and 3.08 to 4.08, respectively in compact type and from 2.41 to 4.25 and 2.75 to 4.33, respectively in semi–compact type during 2002. In 2003, grain mold rating in these panicle type genotypes ranged between 1.36 to 4.66 and 1.00 to 4.29, respectively in loose type; from 1.66 to 4.33 and 1.41 to 3.95, respectively in compact type and from 1.50 to 2.66 and 1.11 to 2.45, respectively in semi–compact type.

Sorghum genotypes had three different types of glume colour *i.e.*, white, brown and red. SGMR–1, SGMR–8, SGMR–9, SGMR–12, SGMR–14, SGMR–24, and 296–B genotypes had white glume colour, ICSB–355, ICSB–370, ICSB–385, ICSB– 394, ICSB– 402, ICSB–403, Bulk–Y and IS–14384 genotypes had brown glume colour and SGMR–3, SGMR–4, SGMR–5, SGMR–6, SGMR–7, SGMR–10, SGMR–11, SGMR–13, SGMR–17, SGMR–21, SGMR–23, ICSB–352, ICSB–362, ICSB–363, ICSB–368, ICSB–380, ICSB–382, ICSB–383, ICSB–391, ICSB–393, ICSB–401, ICSB–407 and PVK–801 genotypes had red coloured glumes. Grain mold rating *i.e.*, FGMR and TGMR in these three different type glume coloured genotypes varied from 3.08 to 4.25 and 2.75 to 4.33, respectively in white glume coloured genotypes, from 2.75 to 4.08 and 2.50 to 4.08, respectively in brown coloured genotypes and from 2.41 to 4.16 and 3.00 to 4.29, respectively in red coloured genotypes during 2002. In 2003, grain mold rating *i.e.*, FGMR and TGMR in these three different types of glume coloured genotypes varied from 1.50 to 4.33 and 1.11 to 4.08, respectively in white glume coloured genotypes, from 1.66 to 2.66 and 1.33 to 2.45, respectively in red coloured genotypes and 1.36 to 4.66 and 1.00 to 4.29, respectively on brown coloured sorghum genotypes.

Sorghum genotypes had three different types of grain colours *i.e.*, white, brown and red. SGMR–1, SGMR–3, SGMR–4, SGMR–5, SGMR–6, SGMR–7, SGMR–8, SGMR–9, SGMR–10, ICSB–352, ICSB–362, ICSB–363, ICSB–368, ICSB–380, ICSB–382, ICSB–394, Bulk–Y, 296B and PVK–801 genotypes had white grain colour, SGMR–23, SGMR–24, ICSB–355, ICSB–383, ICSB–401, ICSB–402 and ICSB–403, genotypes had brown grain colour and SGMR–11, SGMR–12, SGMR–13, SGMR–14, SGMR–17, SGMR–21, ICSB–370, ICSB–385, ICSB–391, ICSB–393, ICSB–407 and IS–14384 genotypes had red coloured grains. Grain mold rating *i.e.*, FGMR and TGMR in these three different types of grain coloured genotypes varied from 2.41 to 4.25 and 3.00 to 4.25, respectively in white grain coloured genotypes, from 3.16, to 4.08 and 3.08 to 4.33, respectively in brown grain coloured genotypes and from 2.75 to 4.16 and 2.50 to 4.29, respectively in red grain coloured genotypes during 2002. In 2003, grain mold rating *i.e.*, FGMR and TGMR in these three different types of grain coloured genotypes varied from 1.75 to 4.66 and 1.45 to 4.29, respectively in white grain coloured genotypes; from 1.58 to 2.66 and 1.11 to 2.16, respectively in brown grain coloured genotypes and from 1.36 to 2.37 and 1.00 to 1.91, respectively in red grain coloured genotypes.

It is seen from aforesaid study that agronomic traits like plant height, glume coverage, panicle type, glume colour and grain colour influenced mold intensity. Murthy (1975), Rao and Rana (1989), Glueck and Rooney (1980) stated that plant traits like panicle shape, plant height, glume structure are important characters for mold resistance. Gangadharan *et al.*, (1978) opined that fully enclosed grains of sorghum by glumes and loose heads are the factors associated with resistance to grain mold. Somani (1992) and Indira and Rana (1977) stressed the importance of compact and longer glumes in selecting sorghum genotypes for resistance to grain molds. Therefore, observations of present study are useful in selecting sorghum genotypes for their resistance to grain mold,

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Table 1 : Effect of agronomic traits of sorghum genotypes on grain mold intensity (Year 2002 and 3003).

Sr. No.	Sorghum Genotypes	Agronomic traits						Mean grain mold intensity							
		Mean plant Height (cm)		Mean glume coverage (%)	Mean panicle morph-ology	Mean glume colour	Mean grain colour	2002				2003			
		2002	2003					FG MR	Rea-ction	TG MR	Rea-ction	FG MR	Rea-ction	TG MR	Rea-ction
1.	SGMR–1	143.33	135	50	SC	White	White	4.25	S	4.25	S	1.83	R	1.5	R
2.	SGMR–3	155	145	75	SC	Red	White	3.25	S	3.5	S	2.08	R	1.75	R
3.	SGMR–4	147.67	140	50	SC	Red	White	3.08	S	3.58	S	2.2	R	1.86	R
4.	SGMR–5	182.33	175	50	SC	Red	White	3.16	S	3.5	S	1.83	R	1.66	R
5.	SGMR–6	185.67	190	50	SC	Red	White	3.08	S	3.91	S	2.66	R	2.45	R
6.	SGMR–7	196.67	184	50	SC	Red	White	3.25	S	3.5	S	1.95	R	1.75	R
7.	SGMR–8	184.67	180	50	SC	White	White	3.16	S	3.33	S	1.86	R	1.75	R
8.	SGMR–9	150	136.67	50	SC	White	White	3.25	S	3.25	S	1.91	R	1.7	R
9.	SGMR–10	203	187.33	25	SC	Red	White	3.91	S	3.75	S	2.08	R	1.75	R
10.	SGMR–11	187.67	178.33	50	SC	Red	Red	4.08	S	4.29	S	2.16	R	1.86	R
11.	SGMR–12	198.33	205	25	SC	White	Red	3.5	S	3.58	S	1.58	R	1.16	R
12.	SGMR–13	191	184.67	50	SC	Red	Red	3.41	S	3.75	S	1.95	R	1.61	R
13.	SGMR–14	194.33	201	50	SC	White	Red	3.08	S	2.75	S	1.5	R	1.36	R
14.	SGMR–17	181.33	166.67	25	SC	Red	Red	4.16	S	4	S	1.66	R	1.5	R
15.	SGMR–21	180.67	169	50	SC	Red	Red	3.41	S	3.33	S	1.83	R	1.33	R
16.	SGMR–23	206.67	205	50	SC	Red	Brown	3.83	S	3.83	S	2.08	R	1.79	R
17.	SGMR–24	156.33	147	25	SC	White	Brown	4.08	S	4.33	S	1.58	R	1.11	R
18.	ICSB–352	201	204	25	SC	Red	White	2.41	R	3	S	1.75	R	1.45	R
19.	ICSB–355	145	145	50	SC	Brown	Brown	3.33	S	3.66	S	2	R	1.75	R
20.	ICSB–362	182	185	25	SC	Red	White	3.41	S	3.5	S	2.41	R	1.97	R
21.	ICSB–363	189.33	198	50	C	Red	White	3.08	S	3.58	S	1.95	R	1.75	R
22.	ICSB–368	190	173.67	25	SC	Red	White	3.16	S	3.25	S	2.45	R	2	R
23.	ICSB–370	187.67	176.67	50	SC	Brown	Red	3.58	S	3.33	S	2.37	R	1.91	R
24.	ICSB–380	187	177.67	50	SC	Red	White	3.16	S	3.41	S	1.83	R	1.61	R
25.	ICSB–382	198.67	200	50	C	Red	White	3.08	S	3.08	S	2.08	R	1.58	R
26.	ICSB–383	207	201	25	C	Red	Brown	3.16	S	3.08	S	1.66	R	1.41	R
27.	ICSB–385	145.67	150	50	SC	Brown	Red	4	S	3.83	S	2.03	R	1.75	R
28.	ICSB–391	148	142.67	50	SC	Red	Red	3	S	3.66	S	2.08	R	1.8	R
29.	ICSB–393	196	200	50	SC	Red	Red	3.41	S	3	S	1.95	R	1.66	R
30.	ICSB–394	181.67	180	50	SC	Brown	White	3.16	S	3.5	S	2.33	R	2.03	R
31.	ICSB–401	148	150	50	SC	Red	Brown	3.91	S	4.08	S	2.33	R	1.66	R
32.	ICSB–402	150	136	50	SC	Brown	Brown	3.75	S	3.83	S	2.66	S	2.16	R
33.	ICSB–403	176.33	167.67	50	SC	Brown	Brwon	3.5	S	3.41	S	1.7	R	1.34	R
34.	ICSB–407	158.33	160	50	SC	Red	Red	3	S	3.25	S	1.91	R	1.58	R
35.	Bulk–Y	129	114	25	L	Brown	White	4.08	S	4.08	S	4.66	S	4.29	S
36.	IS–14384	269	255	90	L	Brown	Red	2.5	R	2.5	R	1.36	R	1	R
37.	296–B	139.67	135	25	C	White	White	3.91	S	4.08	S	4.33	S	3.95	S
38.	PVK–801	182.67	155	50	SC	Red	White	3.33	S	3.08	S	2.08	R	1.66	R
	SE±	3.14	3.19												
	CD at 5 %	8.69	8.85												

Panicle type: C = Compact, SC = Semi- Compact, L = Loose
FGMR : Field grain mold rating

Grain mold reaction: S= susceptible, R=resistant
TGMR: Threshed grain mold rating

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Cellulolytic Activity of Fungal Isolates from the Textile Effluent

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Bioremediation of waste water from textile processing industry is a national need and helps in alleviation of environmental problems. Cellulolytic organisms can be employed for biodegradation of textile effluent due to their ability to produce cellulolytic enzymes. Cellulolytic enzymes are a group of hydrolytic enzymes capable of hydrolyzing cellulose to glucose. The ability of fungi to produce extra cellular cellulolytic enzymes is widespread and these enzyme system have been most extensively studied.
The present communication deals with the screening of fungal isolates for cellulolytic activity for their ability to produce C_x (Endoglucanase) and C_1 (Exoglucanase) using CMC (Carboxymethyl cellulose) and microcrystalline cellulose. The fungi which could grow by utilizing CMC and microcrystalline cellulose as carbon source were *Aspergillus niger, A. flavus, A. oryzae, A. ochraceus, A. terreus, Penicillium chrysogenum, P. expansum, P. funiculosum, P. corylophilum,* Mycelia sterilia, *Trichoderma* sp., *Curvularia andropogonis.* Species of *Aspergillus* and Mycelia sterilia attained maximum growth on the 12th day followed by *Penicillium, Trichoderma,* and *Curvularia* in the medium containing CMC and microcrystalline cellulose.

Keywords : Cellulolytic enzymes, bioremediation, textile effluent, Cellulase activity.

INTRODUCTION

Rapid urbanization and industrialization with an increased awareness about the need for a clean environment have forced industrialist, environmentalist and government to look for efficient, cheap and lasting solutions to waste treatment and recycling (Vijaykumar *et al.*, 2005). The world's ever increasing population and her progressive adoption of an industrial- based lifestyle has inevitably led to an increased anthropogenic impact on the biospere.

In textile production, opportunities exist for the release of potentially hazardous compounds into the ecosystem at various stages of the operation. These pollutants are produced in an effort to improve human standard of living and fashion but ironically, their unplanned intrusion into the environment can reverse the same standard of living by impacting negatively on the environment. Textile effluents can seep into the aquifer and pollute the underground water (Asamudo *et al.* 2005). Cellulose is the most abundant and renewable biopolymer on earth. It is the dominating waste material in the textile effluents. Cellulolytic organisms can be employed for biodegradation of textile effluent due to their ability to produce cellulolytic enzymes (Murai *et al.*, 1998; Hong *et al.*, 2001).

Fungi are recognized for their superior aptitudes to produce a large variety of extracellular proteins, organic acids and other metabolites, and for their capacities to adapt to severe environmental constraints (Lilly and Barnett, 1951; Cochrane, 1958). For example, *Aspergillus niger* is the prototypical fungus for the production of citric acid (Clark, 1962; Lal, 1980; Grewal and Kalra, 1995), homologous proteins (esp. enzymes) and heterologous proteins (Archer *et al.*, 1994; Prasertsan *et al.*, 1997; Wongwicharn *et al.*, 1999; Xu *et al.*, 2000). Moreover, *Phanerochaete chrysosporium* is the model of white-rot fungi for the production of peroxidases (Bumpus *et al.*, 1985; Rodriguez *et al.*, 1999). Beyond the production of such relevant metabolites, fungi have been attracting a growing interest for the biotreatment (removal or destruction) of waste water ingredients such as metals, inorganic nutrients and organic compounds (Field *et al.*, 1993; Palma *et al.*, 1999; Coulibaly, 2002).

In the present study fungal isolates were tested for their ability to produce cellulolytic enzymes. The isolates were assayed for total cellulolytic activity by filter paper assay, carboxymethyl cellulose assay, cotton assay and p-nitrophenyl-β-D-pyranoglucoside (PNPG) method. This entire exercise was a part of initial screening of various isolates for cellulolytic activity to identify the highly active ones for their application in bioremediation of textile effluent.

MATERIAL AND METHODS

Textile effluent and effluent contaminated soil was collected from textile processing industry and the fungal forms were isolates using serial dilution and spread plate method using PDA (Graca *et al.*, 1997).

Fungal isolates were screened and tested for their ability to produce cellulolytic enzyme using carboxymethyl cellulose (CMC) and microcrystalline cellulose (Bradoo *et al.*, 1996).

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The fungal isolates shown maximum growth on petri-plate by utilizing CMC and microcrystalline cellulose were selected and assayed for total cellulolytic activity by filter paper assay (FPA) (Mandel *et al.*, 1976); endoglucanase (Cx) activity by carboxymethyl assay (CMC), cellobiohydrolase (C_1) activity by cotton assay and β-glucosidase activity by using β-nitrophenyl-β-D-pyranoglucosidase (PNPG) method (Rosenberg *et al.*, 1977).

Protein concentration was determined by the method of Lowery *et al.* (1951), using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Fungal isolates shown maximum growth on petri-plate by utilizing CMC and microcrystalline cellulose were the species of *Aspergillus niger, A. terreus, A. ochraceus, A. oryzae, Penicillium chrysogenum, P. expansum, Curvularia andropogonis*, Mycelia sterilia and *Trichoderma* sp. and these isolates were selected and assayed for total cellulolytic activity.

Enzyme profile of fungal isolates for endo-glucanase activity is shown in graph-1. The result showed that C_x activity of fungal isolates *A. ochraceus, A. oryzae, Penicillium chrysogenum, Curvularia andropogonis* and *Trichoderma* sp. appeared in the medium on second day where as for isolates *A. niger, A. terreus, Penicillium expansum* and Mycelia sterilia appeared in medium on the third day after inoculation. Highest C_x activity is shown by *Curvularia andropogonis* on twelfth day (2.716 U/ml), *Trichoderma* sp. on tenth day (2.371 U/ml), Mycelia sterilia (2.290 U/ml) and *P. chrysogenum* (2.229 U/ml) on eleventh day, *Aspergillus niger* on sixth day (2.229 U/ml), *A. oryzae* (1.986 U/ml) and *A. terreus* (1.439 U/ml) on seventh dey, *P. expansum* on eighth day (1.824 U/ml), *A. ochraceus* on fourth day (1.277 U/ml). All the isolates shown decline in the activity after attaining maximum peaks. Isolate *A. ochraceus* disappeared after eleventh day from the medium.

Cellobiohydrolase activity of isolates (Graph- 2) showed that maximum number of isolates appeared in the medium on third day where as *A. terreus, A. ochraceus, A. oryzae* and *Trichoderma* sp. appeared in the medium on second day of inoculation. Maximum C_1 activity is shown by *Trichoderma* sp. on tenth day, *Curvularia andropogonis* (2.279 U/ml) and Mycelia sterilia (0.891 U/ml) on fourteenth day, three isolates *P. chrysogenum* (1.925 U/ml), *P. expansum* (1.641 U/ml) and *A. niger* (0.425 U/ml) on eleventh day, *A. terreus* (0.912 U/ml) on seventh day, *A. ochraceus* (0.506 U/ml) on eighth day, *A. oryzae* (0.445 U/ml) on ninth day. These all isolates shown decline in activity after attaining their peaks. *A. terreus, A. niger* and *A. oryzae* disappeared from the medium after twelfth and thirteenth day.

β-glucosidase activity of fungal isolates is shown in graph-3. The results shown that *A. niger* showed maximum activity (2.981 U/ml) on fourteenth day; *A. ochraceus* (2.375 U/ml), *A. oryzae* (2.286 U/ml), *P. expansum* (2.367 U/ml) on eleventh day; *P. chrysogenum* (2.495 U/ml) and *Curvularia andropogonis* (2.493 U/ml) on ninth day *Trichoderma* sp. (2.454 U/ml) on twelfth day, *A. terreus* (2.333 U/ml) on tenth day, Mycelia sterilia (2.493 U/ml) on thirteenth day. *P. chrysogenum* appeared first in the medium on second day, *A. niger, A. terreus, A. oryzae, Curvularia andropogonis* on third day and *A. ochraceus, P. expansum*, Mycelia sterilia, *Trichoderma* sp. on fourth day after the inoculation.

FPA activity (Graph-4) of fungal isolates shown that *A. niger* showed maximum activity (2.339 U/ml) on twelfth day, *A. terreus* (2.282 U/ml) and *A.ochraceus* (1.641 U/ml) on eighth day, *A. oryzae* (2.231 U/ml) and *P. expansum* (1.824 U/ml) on tenth day, *P. chrysogenum* (2.300 U/ml) on eleventh day, *Curvularia andropogonis* (1.743 U/ml) on thirteenth day, Mycelia sterilia (1.439 U/ml) and *Trichoderma* sp. (1.541 U/ml) on fourteenth day. Isolates *A. niger, A. ochraceus, A. oryzae, P. expansum* and *Curvularia andropogonis* after attaining peak first shown decline in the activity and then disappeared from the medium.

Extracellular protein activity of all fungal isolates is shown in graph- 5. The results shown that *P. chrysogenum* showed maximum protein activity (2.59 mg/ml) on twelfth day; *A. niger* (2.54 mg/ml), *A. ochraceus* (1.42 mg/ml), *P. expansum* (1.61 mg/ml), Mycelia sterilia (1.52 mg/ml) on fourteenth day; *Trichoderma* sp. (2.92 mg/ml) and *A. oryzae* (1.60 mg/ml) on thirteenth day, *Curvularia andropogonis* (1.52 mg/ml) on twelfth day, *A. terreus* (1.72 mg/ml) on ninth day.

In present investigation attempt was made to select efficient cellulase producers which can be used in biodegradation processes. The results showed that all the chosen organisms possessed a complete cellulolytic enzyme system and showed higher cellulolytic activity. *Curvularia andropogonis* and *Trichoderma* sp. possessed highest C_x and C_1 activity; *A. niger* possessed the highest β-glucosidase and FPA activity, other cultures showed appreciable quantities of all the enzymes.

The present work is being supported by the work of Vaidya (1992) where six fungal strains *A. niger, A. fumigatus, Polystictus versicolor, Ptychogaster* sp., *Sporotrichum pulverulentum, Trichoderma viride* were studied for cellulase enzyme activity. For determination of fungal cellulolytic activity standard Mandel and Weber's basic medium with Walseth cellulose as sole carbon source is used and isolates were assayed for total cellulolytic activity by filter paper assay, C_x activity by CMC assay, C_1 activity by cotton assay and β-glucosidase activity by using (PNPG) method. In our study also the cellulase enzyme activity of six fungal strains was studied on a comparative basis over a period of 14 days in order to select the efficiant cellulolytic organisms. Out of all fungal strains *Aspergillius niger* showed highest β-glucosidase activity and other five fungal isolates showed good quantities of all the enzymes.

Kader and Omar (1998), Kader *et al.* (1999) isolated cellulolytic fungi from soil and assayed for cellulolytic activity. In their study isolates of *Aspergillus* and *Trichoderma* showed high cellulolytic filter paper activity, carboxymethyl cellulose and β-glucosidase activities. Picart *et al.* (2007) isolated fungal strains from subtropical soil and maximum cellulase activity was produced by *Penicillium* sp. CR-316.

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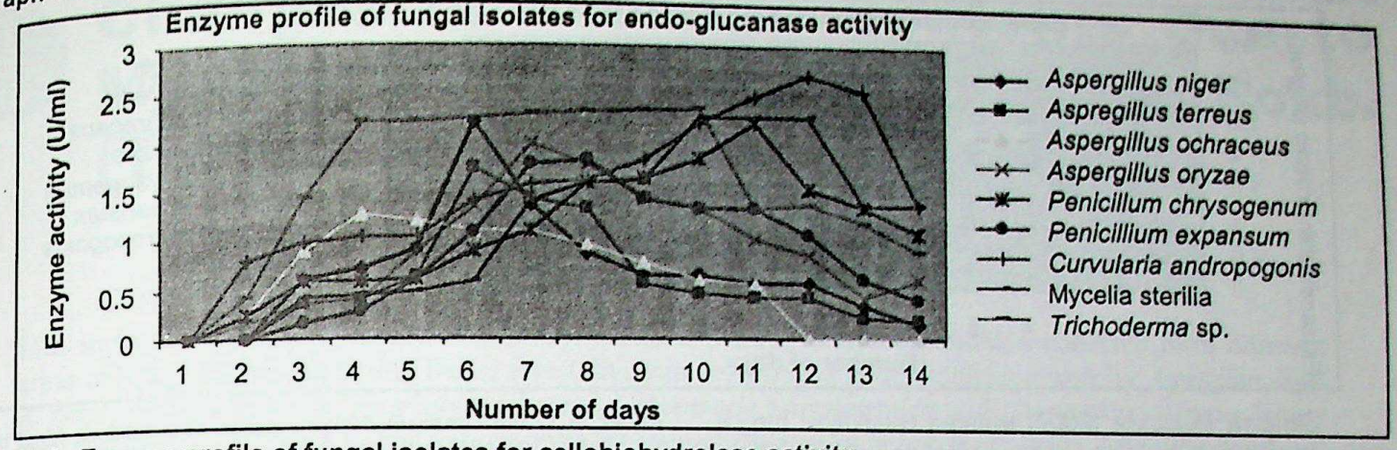
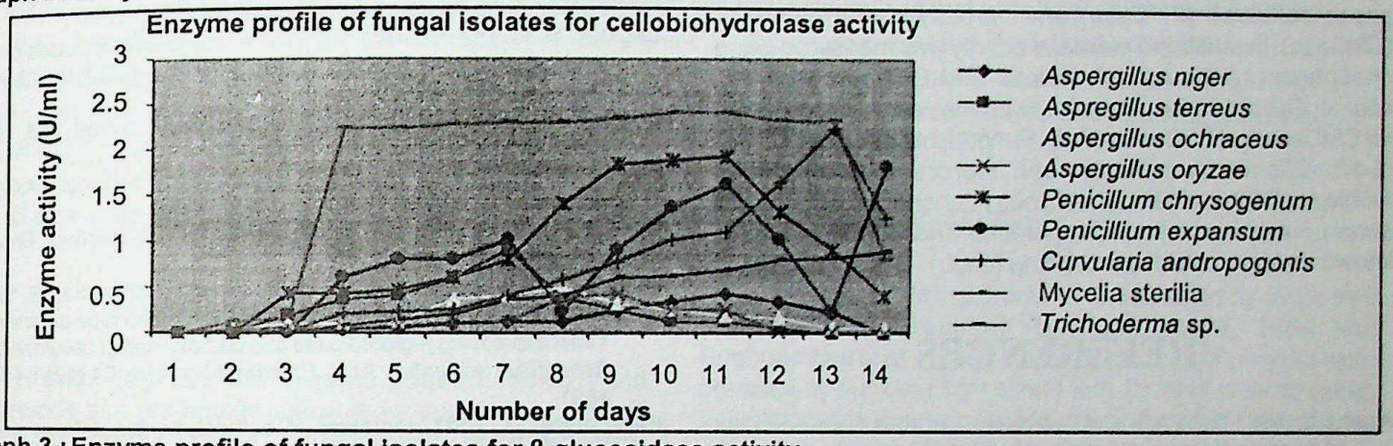
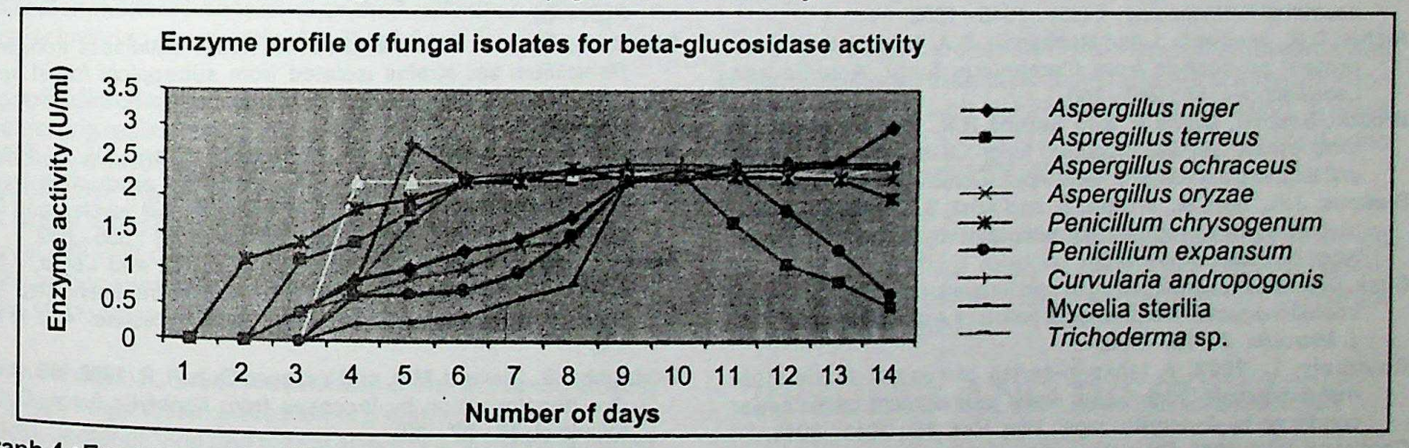
Graph 1 : Enzyme profile of fungal isolates for endo-glucanase activity.



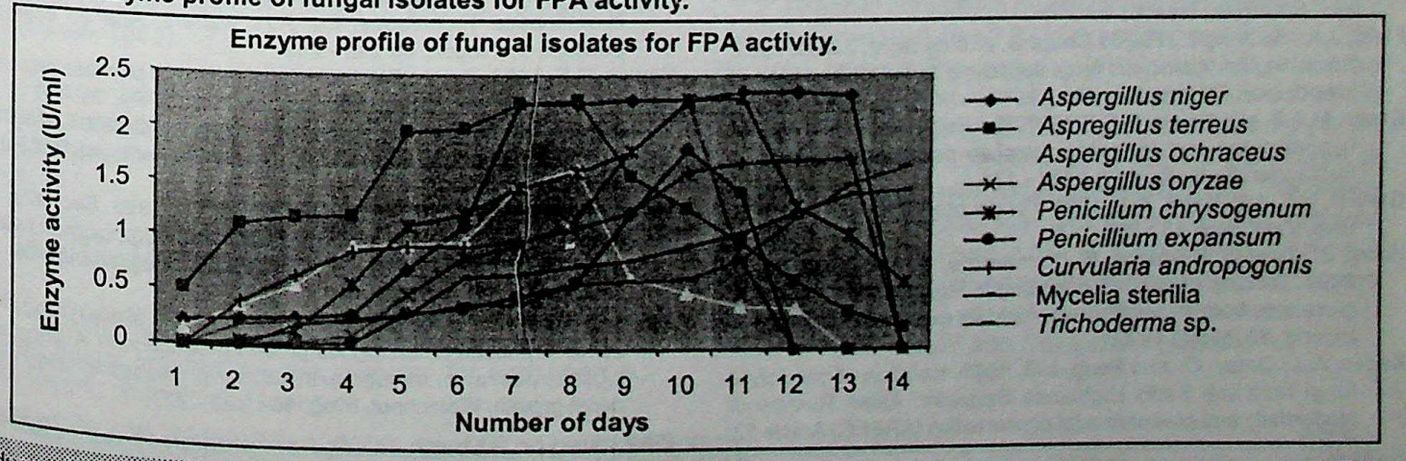
Graph 2 : Enzyme profile of fungal isolates for cellobiohydrolase activity.



Graph 3 : Enzyme profile of fungal isolates for β-glucosidase activity.



Graph 4 : Enzyme profile of fungal isolates for FPA activity.



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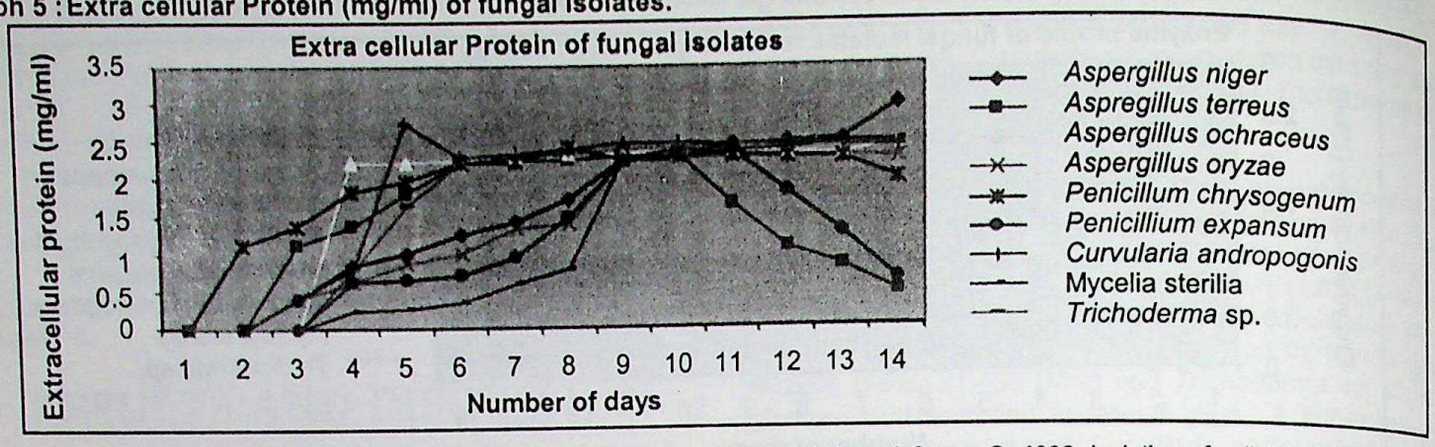
Graph 5 : Extra cellular Protein (mg/ml) of fungal isolates.



Similarly Peciulyte (2007) isolated cellulolytic fungal strains from paper waste and assayed for two enzymes- filter paper cellulase (FPCase) and carboxymethyl cellulase (CMCase). Quantitative cellulase activity was measured using phosphoric acid swollen cellulose and CMC as a carbon source. Out of 139 fungal strains twelve isolates were selected for CMCase and FPCase activity. Fungal isolates *A. niger* DPK-cl-12, *Gliomastix murorum* var. *murorum* (*Acremonium murorum*) DPK-cl-44 and *Stachybotrys chartarum* DPK-cl-111 shown maximum activity compared to *Trichoderma* which is known as high cellulose producing fungi.

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Changes in Phenolic Contents Due to Mycoflora of *Diospyros melanoxylon* Roxb. (Tendu) Leaves During Storage

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Leaf surface mycoflora of *Diospyros melanoxylon* Roxb. (tendu) in stored condition collected from different areas of Durg, Rajnandgaon and Bastar forest divisions were found to be dominated over by *Aspergillus niger. A. flavus, Curvularia lunata, Pestalotia machrotricha, Mammaria echinobotryoides* and *Chaetomium globosum.* These fungal isolates were found to bring about quite substantial changes in phenolic contents. *Aspergillus flavus* and *Mammaria echinobotryoides* were found to be responsible for increase in the level of total phenols well as ortho dihydric phenols, while all the six fungal isolates were found responsible for depletion in tannin content. *Chaetomium globosum* was found to cause about 80% fall in phenolic contents favouring the growth of other molds due to increased susceptibility of the host causing damage to the stored leaves and affecting their self life and commercial value.

Keywords : Phenolic contents. Storage mycoflora, Forest divisions, Fungal isolates.

INTRODUCTION

Fungi degrade the organic substances to yield compounds that are readily utilizable for their own nutritional and metabolic requirements. The leaf surface mycoflora utilize and exploit nutrients according to their utilization efficiencies there by lowering commercial quality and self life of the leaves.

Total and ortho dihydric phenols play an important role in conferring resistance to the tissues against fungal infection. Phenols are oxidized to highly reactive quinones, which are quite effective inhibitors of enzymes having sulphydryl group and form a lining over cell wall of the host, making it resistant against cell wall degrading enzymes secreted by pathogens during pathogenesis (Rubin and Artisikhovskaya, 1964; Friend, 1980, 1981; Santos and Valter, 2004; Adam *et al.*, 2006).

The tannin, one of the phenolic content of tendu leaves, also plays major role in protection of plants against herbivores, insects, bacterial, viral and fungal attack due to their astringent effect and by inactivating enzymes of insects, bacteria and fungi (Swain, 1965).

Present investigation deals with the quantitative estimation of changes in phenolic contents mainly caused by storage mycoflora of *Diospyros melanoxylon* Roxb. (tendu) leaves.

MATERIAL AND METHODS

Healthy tendu leaves and those infested with six different fungal organisms were analysed for total phenols, ortho dihydric phenols and tannins.

Total phenols in extracts were estimated by employing Folin–ciocalteu reagent (Bray & Thorpe, 1954 and Raghunathan *et al.*, 1966). Water extractions of phenols were obtained by blending 1gm of leaf with 20 ml of distilled water. Folin–ciocalteu reagent used in present analysis was diluted with equal volume of distilled water just before use.

Ortho dihydric phenols were extracted in the same way as above and were estimated quantitatively following Jhonson & Schaal (1951), with colorimetric method using Arnows reagents, 0.5 N HCl and 01 N NaOH solutions. Total and ortho dihydric phenols were expressed as catechol equivalents.

Total tannins were estimated quantitatively following Association of Offical Analytic Chemistry (AOAC, 1990), titration method using Indigo solution and Potassium permanganate solution.

RESULTS AND DISCUSSION

Phenolic contents in healthy tendu leaves were 0.89 ± 0.00 (total phenol), 0.40 ± 0.02 (o.d. phenol) and 3.23 ± 0.08% DM (total tannin). The increase in total phenol and ortho dihydroxy phenol content, per gram of leaves, were 0.46 mg (51.69% gain) & 0.11 mg (27.50% gain) and 0.36 mg (40.44 % gain) & 0.13 mg (32.50% gain) after fifteen days of incubation, infested with *Aspergillus flavus* and *Mammaria echinobotryoides* respectively. The fall in phenolic contents for infested leaves were up to 60 - 70 % in case of total phenol and 60 - 82% in case of ortho dihydric phenol when, leaves were infested with *Aspergillus niger, Curvularia lunata, Pestalotia machrotricha* and *Chaetomium globosum.*

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TOTAL PHENOL, ORTHO DIHYDRIC PHENOL AND TOTAL TANNIN CONTENT IN HEALTHY AND FUNGAL INFESTED TENDU LEAVES AFTER 15 DYAS OF INCUBATION PERIOD

TENDU LEAF SAMLE	TOTAL PHENOL			ORTHO DIHYDRIC PHENOL			TOTAL TANNIN		
Tendu leaves infested with	mg/gm	Loss/Gain mg/gm	% Loss /Gain	mg/gm	Loss/Gain mg/gm	% Loss /Gain	% DM basis	Loss/Gain % DM basis	% Loss
Aspergillus niger	0.32 ± 0.00	–0.56 ± 0.00	–62.92%	0.11 ± 0.01	–0.29 ± 0.02	–72.50%	1.49 ± 0.06	–1.74 ± 0.01	–53.87%
Aspergillus flavus	1.35 ± 0.02	+0.46 ± 0.02	+51.69%	0.52 ± 0.00	+0.11 ± 0.02	+27.50%	1.36 ± 0.06	–1.87 ± 0.01	–57.89%
Curvularia lunata	0.29 ± 0.02	–0.46 ± 0.02	–67.42%	0.08 ± 0.01	–0.32 ± 0.01	–80.0%	1.03 ± 0.10	–2.20 ± 0.04	–68.11%
Pestalotia machrotricha	0.36 ± 0.01	–0.53 ± 0.01	–59.55%	0.15 ± 0.02	–0.25 ± 0.02	–62.50%	1.58 ± 0.11	–1.65 ± 0.03	–51.08%
Mammaria echinobotryoides	1.25 ± 0.00	+0.36 ± 0.00	+40.44%	0.54 ± 0.00	+0.13 ± 0.02	+32.50%	0.70 ± 0.04	–2.53 ± 0.01	–78.33%
Chaetomium globosum	0.25 ± 0.02	–0.64 ± 0.02	–71.91%	0.07 ± 0.00	–0.33 ± 0.02	–82.50%	0.62 ± 0.06	–2.61 ± 0.01	–80.80%
Healthy tendu leaves	**0.89 ± 0.00**			**0.40 ± 0.02**			**3.23 ± 0.08**		

+ or – = Gain or Loss **Data presented is mean of 3 replicates**

ANALYSIS OF VARIANCE FOR TWO WAY ANOVA

Source of variation	Sum of Squares (SS)	Degree of Freedom (df)	Mean Squares (MS)	F–ratio	5%F–limit	1% F–limit
Between incubation period (Total phenol)	6575.07	2	3287.53	110.57	4.10*	7.56*
Between species	1835.24	5	367.04	12.34	3.33*	5.64*
Error	279.35	10	29.73			
Total	8707.66	17				
Between incubation period (Ortho dihydirc phenol)	6736.44	2	3368.22	38.36	4.10*	7.56*
Between species	3299.22	5	659.84	7.51	3.33*	5.64*
Error	877.9	10	87.79			
Total	10913.56	17				
Between incubation period (Total tennin)	4862.12	2	2431.06	25.44	4.10*	7.56*
Between species	2238.37	5	447.67	4.68	3.33*	5.64*
Error	955.38	10	95.53			
Total	8055.87	17				

*** = Significant**

Similarly depletion in tannin contents were –1.74% DM (53.87% loss), –1.87 % DM (57.89% loss), –2.20 % DM (68.11 % loss), –1.65 % DM (51.08 % loss), –2.53 % DM (78.33 % loss) and –2.61 % DM (80.80 % loss) after fifteen days of incubation, when leaves were infested with *A. niger, A. flavus, C. lunata, P. machrotricha, M. echinobotryoides* and *C. globosum* respectively.

A. niger, C. globosum and *P. machrotricha* appeared to be responsible for maximum lowering of phenolic compounds. On the other hand, *A. flavus* and *M. echinobotryoides,* which caused comparatively lesser damage were found to be responsible for increase in the level of total as wall as ortho dihydroxy phenol contents but decrease in the level of tannins considerably. Since resistance of the stored leaves against fungi is largely due to total & ortho dihydroxy phenols, their increase restricted the fungal invasion while their depletion made leaves more susceptible.

The variation in the percentage loss / gain of phenolic contents due to incubation period is significant at 1% level. The table also shows that the variation in percentage loss of total phenol & o.d. phenol due to fungal species is significant at 1% level and in case of total tannin at 5% level.

The natural durability of many plant species is due to the toxicity of phenolics to infesting microorganisms (Jung and Fahey, 1983). Increase in phenolic contents in relation to resistance has been reported in Gram infected with *Ascochyta rabiei* (Vir and Grewak, 1974), Brinjal infected with *Verticillium dahliae* (Sivaprakasan *et al.*, 1974), Pearl millet infected with *Sclerospora graminicola* (Arya *et al.*, 1978), *Cyperus rotundus* leaves infected with rust fungi (Purohit *et al.*, 1979), Coriander seed infected with *Protomyces* sp. (Naqui, 1982) and coriander and groundnut seeds infected with *A. niger, A. flavus* and *A. fumigatus* (Bindu, 1997).

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Degradation of plant tissues and their cell walls occurs as a result of the activity of a large number of microorganisms. It has been found that large number of microbes co–exist in almost all natural environments (Chandrol, 2002; Nair *et al.*, 2008). However, microorganisms suffer from substrate inhibition at higher concentration of phenol, by which their growth is inhibited (Prieto *et al.*, 2002; Borghei and Hosseini, 2004; Mohd Tuah, 2006).

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In-vitro Evaluation of Botanicals Against *Sclerotium rolfsii* Sacc. Inciting Collar Rot of Soybean

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Results revealed that all the botanicals tested exhibited fungistatic /antifungal activity and significantly inhibited radial mycelial growth as well as sclerotial production of *S. rolfsii* over untreated control. However, leaf extracts of *Datura stramonium* (Datura) and *Annona squamosa* (custard apple) were at par and recorded highest average mycelial inhibition, respectively of 58.35 and 58.17 per cent and average sclerotial inhibition, respectively of 52.27 and 50.30 per cent. This was followed by the leaf extracts of *Lawsonia innermis* (mehandi), *Ipomea carnea* (beshram) and *Jatropha curcas* (jatropha) which recorded average mycelial inhibition, respectively of 53.83, 53.67 and 52.41 per cent and average sclerotial inhibition respectively of 45.47, 42.70 and 39.88 per cent. Rest of the leaf extracts tested were also found antifungal and caused significant inhibition of mycelial growth and sclerotial production of the test pathogen. Antifungal activity of the test botanicals was increased with increase in their concentrations.

Keywords : Soybean, botanicals, *Sclerotium rolfsii,* fungistatic, antifungal, Mycelium sclerotia, inhibition.

INTRODUCTION

Soybean (*Glycine max* L. Merill), popularly known as Golden bean or Poor man's meat, is one of the grain legumes popularly grown worldwide. The world's demand of about 40% edible vegetable oil has been meet only through soybean. Among the conventional oilseed crops, Soybean, ranks 3rd followed by groundnut and rapeseed mustard. During recent years, Soybean cultivation has attained a big stride in country, espceically in the states of Maharashtra, Madhya Pradesh, Andhra Pradesh, Karnataka and Rajasthan. More than 100 pathogens have been reported in infecting soybean and tend to cause globally seven million tones of yield losses. Of the major disease of soybean, collar rot incited by *Sclerotium rolfsii* Sacc. is becoming a major threat to the cultivation of soybean. The pathogen (*S. rolfsii*) infect soybean during early crop growth stages and thereby potentially minimize final productive plant population per unit area, resulting ultimately into accountable yield losses of about 25 to 30 per cent. The pathogen, being soil borne with wide host range, is difficult and uneconomical to manage with chemicals/fungicides. With an aim to find out suitable and eco-friendly disease management strategy, present *in vitro* studies were conducted to find out the antifungal potential of 14 botanical leaf extracts against *S. rolfsii.*

MATERIAL AND METHODS

Plant leaf extracts of 14 botanicals *viz.*, Neem (*Azadirachta indica*), Tulshi (*Ocimum sanctum*), Eucalyptus (*Eucalyptus globulus*), Datura (*Datura stramonium*), Jatropha (*Jatropha curcas*), Periwinkle (*Vinca rosea*), Custard apple (*Annona squamosa*), Ghaneri (*Lantana camara*), Ashok (*Polyalthia longifolia*), Golden duranta (*Duranta* spp.), Beshram (*Ipomea carnea*), Parthenium (*Parthenium hysterophorus*), Merigold (*Tagetes erecta*) and Mehandi (*Lawsonia inermis*) were evaluated *in vitro* against *S. rolfsii,* applying poisoned food technique (Grover and Moore, 1962, Alice, 1984). Leaf extract of the test botanicals were prepared by grinding in mixture-cum-grinder. Take 100 gm washed leaves of each plant species in 100 ml distilled water and macerate was filtered through double layered muslin cloth and further through Whatmann No. 1 filter paper. The final clear extracts obtained were of 100% concentration which were evaluated @ 10 and 20 per cent. Potato dextrose agar was used as basal culture medium. An appropriate quantity of each leaf extract (100%) was separately mixed thoroughly with PDA in conical flask to obtain desired concentration and autoclaved at 15 lbs / cm^2 pressure for 15-20 min. Autoclaved and cooled (40°C) PDA amended with leaf extract was poured (15-20 ml / plate) into sterile glass Petriplates (90 mm dia) and allowed to solidity at room temperature. Upon solidification of PDA, all plates were aseptically inoculated by placing in the centre as 5 mm mycelial disc obtained from a week old pure culture of *S. rolfsii.* A suitable untreated control was also maintained. Treated and untreated plates were incubated at 27 ± 2°C, till untreated plates were fully covered with mycelial growth of the test pathogen. The study was conducted applying the design, CRD and all the treatments were replicated twice. Observations on radial mycelial growth and number of sclerotia produced were recorded and per cent inhibition of mycelial growth and sclerotial production over untreated control were computed applying the formula given by Vincent (1927).

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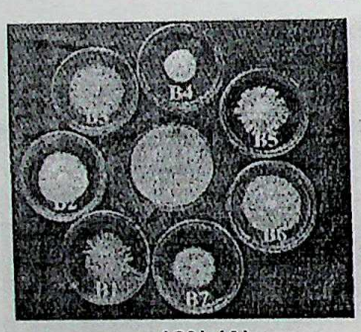
Table 1 : *In vitro* effect of botanicals on mycelial growth and inhibition of *S. rolfsii*.

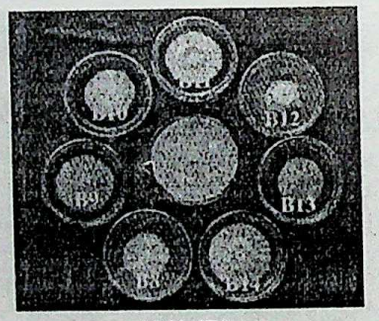
Botanicals	Colony dia.* (mm) at		Avg. colony dia. (mm)	% Inhibition* at		Avg. Inhibition (%)
	10%	20%		10%	20%	
Neem (*A. indica*)	55.50	52.40	53.95	38.33 (38.25)	41.78 (40.27)	40.06
Tulsi (*O. sanctum*)	59.20	54.30	56.75	34.22 (35.80)	39.66 (39.03)	36.94
Eucalyptus (*E. globulus*)	61.50	54.25	57.88	31.67 (34.24)	39.72 (39.07)	35.70
Datura (*D. stramonium*)	48.90	25.70	37.30	45.66 (42.51)	71.44 (57.67)	58.55
Jatropha (*J. curcas*)	52.15	33.50	42.80	42.05 (40.43)	62.77 (52.40)	52.41
Periwinkle (*V. rosea*)	58.65	38.90	48.78	34.83 (36.17)	56.77 (48.89)	45.80
Custard apple (*A. squamosa*)	49.20	26.41	37.65	45.33 (42.32)	71.00 (57.41)	58.17
Ghaneri (*L. camara*)	53.45	48.70	51.08	40.61 (39.59)	45.89 (42.64)	43.25
Ashoka (*P. longifolia*)	65.10	56.90	61.00	27.66 (31.73)	36.77 (37.33)	32.22
Golden duranta (*Duranta* spp.)	61.25	49.50	55.38	31.94 (34.41)	45.00 (42.13)	38.47
Besharam (*I. carnia*)	50.50	32.90	41.70	43.89 (41.49)	63.44 (52.80)	53.67
Parthenium (*P. hysterophorus*)	64.20	40.75	52.48	28.66 (32.37)	54.72 (47.71)	41.69
Marigold (*T. erecta*)	57.70	42.05	49.83	36.00 (36.86)	53.28 (46.89)	44.69
Mehandi (*L. inermis*)	53.20	29.90	41.55	40.89 (39.74)	66.77 (54.80)	53.83
Control	90.00	90.00	90.00	00.00 (04.06)	00.00 (04.06)	00.00
S.E. ±	00.37	00.26	--	00.25	00.17	--
C.D. (P = 0.05)	01.12	00.79	--	00.75	00.51	--

* : Mean of two replications, Figures in parentheses are arcsine values.

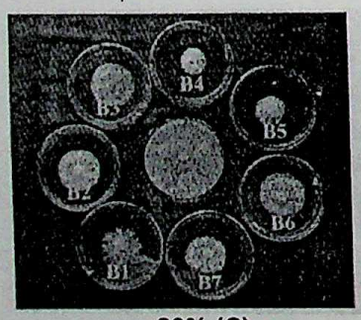
PLATE - 1



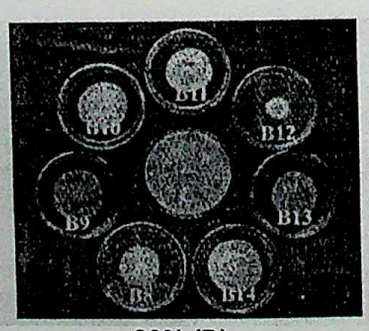
10% (A)



10% (B)



20% (C)



20% (D)

***In vitro* effect of botanicals @ 10% (A and B) and 20% (C and D) on mycelial growth of *S. rolfsii*.**

B1) Neem
B2) Tulsi
B3) Eucalyptus
B4) Datura
B5) Jatropha
B6) Periwinkle
B7) Custard apple
B8) Ghaneri
B9) Ashoka
B10) Golden duranta
B11) Besharam
B12) Parthenium
B13) Marigold
B14) Mehandi
C) Control

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Table 2 : *In vitro* effect of botanicals on sclerotial production and inhibition of *S. rolfsii*.

Botanicals	Sclerotial production* at				Avg. Sclero. prod.	% Inhibition* at		Avg. Inhib. %
	10%		20%			10%	20%	
	O.V.	$\sqrt{X + 0.5}$	O.V.	$\sqrt{X + 0.5}$				
Neem (*A. indica*)	149.50	12.25	88.00	09.41	118.75	19.13 (25.94)	48.82 (44.82)	33.98
Tulsi (*O. sanctum*)	152.50	12.37	90.54	09.54	121.50	17.78 (24.94)	47.37 (43.49)	32.58
Eucalyptus (*E. globulus*)	151.00	12.31	92.50	09.64	121.75	18.59 (25.54)	46.22 (42.83)	32.41
Datura (*D. stramonium*)	107.00	10.37	65.00	08.09	86.00	43.32 (40.58)	62.21 (52.06)	52.27
Jatropha (*J. curcas*)	147.00	12.50	70.50	08.43	108.75	20.76 (27.10)	59.00 (30.19)	39.88
Periwinkle (*V. rosea*)	146.00	12.10	76.50	08.78	111.25	21.29 (27.48)	55.53 (48.17)	38.41
Custard apple (*A. squamosa*)	110.50	10.54	68.50	08.30	89.50	40.43 (39.48)	60.16 (50.86)	50.30
Ghaneri (*L. camara*)	146.00	12.10	86.50	09.33	116.25	21.29 (27.48)	49.70 (48.17)	35.50
Ashoka (*P. longifolia*)	159.50	12.67	95.50	09.80	127.50	14.02 (21.98)	44.48 (41.83)	29.25
Golden duranta (*Duranta* spp.)	150.00	12.27	88.00	09.41	119.00	19.40 (26.13)	48.32 (44.32)	33.86
Besharam (*I. carnia*)	129.00	11.38	77.50	08.83	103.25	30.46 (33.49)	54.94 (48.12)	42.70
Parthenium (*P. hysterophorus*)	149.50	12.25	87.50	09.38	118.50	19.40 (26.13)	49.11 (44.49)	34.26
Marigold (*T. erecta*)	147.00	12.15	85.00	09.25	116.00	20.76 (27.10)	50.57 (45.32)	35.67
Mehandi (*L. inermis*)	122.50	11.09	74.00	08.63	98.25	33.96 (35.64)	56.97 (49.01)	45.47
Control	185.50	13.67	172.00	13.13	178.75	00.00 (04.06)	00.00 (04.06)	00.00
S.E. ±	– –	00.04	– –	00.06	– –	00.28	00.45	– –
C.D. (P = 0.05)	– –	00.11	– –	00.19	– –	00.84	01.36	– –

* : Mean of the two replications Figures in parentheses are arcsine values

OV : Original value (no. of sclerotia) $\sqrt{X + 0.5}$: Square root transformed value.

RESULTS AND DISCUSSION

(A) Mycelial growth and inhibition : Results (Table 1) revealed that all the botanicals tested caused significant inhibition of mycelial growth (PLATE–1) as well as sclerotial production of the test pathogen over untreated control. Radial mycelial growth recorded with all the test botanicals at 10 and 20 per cent concentrations was ranged from 48.90 to 65.10 mm and 25.70 to 56.90 mm, respectively with an average in the range of 37.30 to 61.00 mm. However, least average radial mycelial growth was record with *D. stramonium* (37.30 mm) and *A. squamosa* (37.65) which were at par. This was followed by *L. inermis* (41.55 mm) and *T. erecta* (49.83 mm). All the botanicals significantly inhibited mycelial growth of the test pathogen. Mycelial growth inhibition with all the botanicals at 10 and 20 per cent conc. was ranged from 27.66 to 45.66 and 36.77 to 71.44 per cent, respectively with average inhibition in the range of 32.22 to 58.55 per cent. However, significantly highest average mycelial inhibition was recorded with *D. stramonium* (58.55%) and *A. squamosa* (58.17%) which were at par. This was followed by *L. inermis* (53.83%), *I. carnea* (53.67%) and *J. curcas* (52.41%). Rest of the botanicals recorded less than 50 per cent average mycelial inhibition and it was ranged from 32.22 to 45.80 per cent.

(B) Sclerotial Production and Inhibition : Results (Table 2) revealed that all the botanicals tested significantly inhibited sclerotial production of the test pathogen. The number of sclerotia recorded with the test botanicals at 10 and 20 per cent concentration were ranged from 107.00 to 152.50 and 65.00 to 92.50, respectively with average sclerotia in the range of 86.00 to 127.50. However, least average number of sclerotia were produced with *D. stramonium* (86.00) and highest were produced with *P. longifolia* (127.50). All the botanicals tested caused significant inhibition of sclerotial production of the test pathogen and it was ranged from 14.02 to 43.32 per cent at 10 per cent conc. and 44.48 to 62.21 per cent at 20 per cent conc. Average sclerotial inhibition recorded with test botanicals was ranged from 29.25 to 52.27 per cent. However, significantly highest average sclerotial inhibition was recorded with *D. stramonium* (52.27%), followed by *A. squamosa* (50.30%), *L. inermis* (45.47%) and *I. carnea* (42.70%). Rest of the botanicals recorded comparatively minimum inhibition less than 40 per cent and it was ranged from 29.25 to 39.88 per cent. The percentage inhibition of the mycelial growth and sclerotial production of the test pathogen was found to increased with increase in their concentrations. The fungistatic / fungitoxic activity of the plant leaf extracts against *S. rolfsii* might be due to the presence of the specific antifungal compounds like phenols, tannins, resinous and non–volatile substances in the botanicals tested.

Result of the present study on antifungal effects of the botanical tested against *S. rolfsii* are in consonance with those reported earlier by several workers. Leaf extracts of *A. indica*,

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V. rosea, A. squamosa, D. stramonium, P. hysterophours, L. camara, L. inermis etc. were reported antifungal against *S. rolfsii, M. phaseolina, R. solani, Colletotricium* spp. and *Fusarium* spp, earlier by several workers (Alice, 1984; Ravichandar, 1987; Sobti *et al*, 1995; Pani and Patra, 1997; Meena *et al*., 2000; Kaushal *et al*, 2003; Sesha Kiran *et al*, 2006 and Suryawanshi *et al*, 2007).

CONCLUSION

From the present study it can be concluded that botanicals *viz., D. stramonium, A. squamosa, L. inermis, I. carnea, I. erecta* and *P. hysterophorous,* found antifungal against *S. rolfsii* can further be exploited commercially for management of soil-borne diseases including collar rot of soybean. However, further studies needs to be undertaken for detection of antifungal compounds and confirmation of antifungal activities of the botanicals tested in present study.

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Biodiversity of Arbuscular Mycorrhizal (AM) Fungi Associated with Cultivated Medicinal Plants

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Survey for association of AM fungi in cultivated medicinal plants showed that most of the plant species had prominent AM fungal colonization in the form of fungal structures, vesicals and arbuscules. Four medicinal plants (*Rauwolfia serpentina, Coleus aromaticus, Adathoda vasica* and *Andrographis peniculata*) were identified as effective colonizinging species. *Rauwolfia serpentina* showed maximum (72%) colonization and highest spore number (260 ± 1.21) among the medicinal plants taken in the study. Twelve species of AM fungi belonging to genus *Glomus, Acaulospora. Gigaspora, Sclerocystis, Scutellospora* and *Entrophospora* were isolated. *Rauwolfia serpentina* showed maximum species richness (11 species) followed by *Coleus aromaticus* (10), *Adathoda vasica*(9) and *Andrographis peniculata* (7) respectively. *Glomus aggregatum, G. macrocarpum, G. fasciculatum, G. mosseae, Aculospora appendiculota* and *Scutellospora* sp. were dominating with 100% frequencies. *Acaulospora laevis* and *Gigaspora* sp. (60%) came in moderate frequency range followed by less frequency of *Glomus constrictum, G. geosporum, Entrophospora* sp. (40%) and *Sclerocystis* sp. (10%) respectively. *G. fasciculatum, G.mosseae* and *Acaulospora appendiculata* showed maximum densities (3.0) among the AMF species recorded. Genus *Glomus* emerged as a dominant member among the AM fungi.

Keywords : Biodiversity, AM fungi, medicinal plants, species richness, frequency, density, *Glomus.*

INTRODUCTION

World is endowed with a rich wealth of medicinal plants. Herbs have always been the principal form of medicine in India. Medicinal value in the plants depends upon the availability of nutrients in soil where they are growing. AM fungi play an important role in improving the nutrients uptake and growth of plant species. They are of immense value for being a transporter and carrier of various minerals like P, Zn, Mn, Cu and Al from soil to the host plant (Sampath Kumar *et al.*, 2006 and Ratageri *et al.* 2006). Mycorrhization may also influences synthesis of alkaloids in such plants increasing their medicinal values (Mishra *et al,* 2006 and Sharma *et al.*, 2007 a).

The study site Durg is situated in central plains of Chhattisgarh, having tropical climate. The state has been declared as Herbal state. After the establishment of Chhattisgarh Rajya Vanoushadhi board, many farmers are shifted to cultivation of medicinal plants as alternative crops. In the present study, an attempt was made to assess biodiversity of AM fungi in cultivated medicinal plants of Durg dist.

MATERIAL AND METHODS

Based on the data available from Chhattisgarh Rajya Vanoushadhi Board, Raipur, for cultivation of Medicinal plants in the environment of Chhattisgarh, particularly in Durg dist., twelve commonly cultivated medicinal plants were selected for the study. Most of the selected plants were of annual herbaceous habit and cultivated by either seeds or vegetative mode of propagation.

Random selected plants were brought to laboratory along with rhizosphere soil in polythene bags with proper labeling. Root samples were carefully washed, cleaned in 10%KOH and stained with 0.05% trypan blue in lactophenol (Phillips and Hayman 1970). The stained root segments were observed for mycorrhizal structure under light microscope at high magnification. The root pieces were counted containing vesicle, arbuscles and hyphal structures for determination of colonization percentage by the following formula-

$$\text{Per cent root colonization} = \frac{\text{No.of root segment infected}}{\text{Total no.of root segment studied}} \times 100$$

AM fungi exist in soil as chlamydospore. The spores were isolated from each soil sample using wet sieving and decanting technique (Gardmann and Nicoloson, 1963) and studied for their morphological characters. Based on the spore characters the fungal genera and species were identified. The identification of individual mycorrhizal species was done by taxonomic key provided by www.invam.caf.wvu.edu, Gupta (1995) and Gupta *et al.* (1997).

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The identified spores were counted microscopically for the calculation of percentage frequency and density by the method described by Phillips (1959).

RESULTS AND DISCUSSION

The root samples of each selected plant were analyzed for per cent colonization. Most of the plant species had prominent AM fungal colonization in the form of fungal structures, vesicles and arbuscules (Table -1). It was observed that percentage of mycorrhizal infection varied with the plant species with a range of 12 to 72% in *Becopa monnieri* (Brahmi) and *Rauwolfia serpentina* (Sarpgandha) respectively. The mycorhhizal spores were also found in variable numbers. Maximum spore population was observed in the rhizosphere soil of *Rauwolfia serpentina* (260 ± 1.21) followed by *Coleus aromaticus* (187 ± 1.45), *Adathoda vasica* (110 ± 1.34) and *Andrographis peniculata* (97 ± 1.37) and least with *Piper longum* (25 ± 1.22). No definite relationship could be observed between AM colonization and spore population. The differential ranges of root colonization and spore load exhibit differential responses of host plants towards mycorrhization. The lower ranges of infection might be due to amendment of cultivation fields with agrochemicals and organic manure in order to get good yields. Dependency of symbiotic effectiveness of AM fungi on their preference of soil type and host plant specificity have also been reported by Sagar and Roy (2005); Ratageri *et al.* (2006) and Sharma *et al.* (2007 b).

Rauwolfia serpentina, Coleus aromaticus, Adathoda vasica and *Andrographis peniculata* were found as effective colonizing species. Predominance of AMF (species richness) in their corresponding hosts was further analyzed in these species. The data in Table- 2 revealed that 12 AMF endophytes representing six species of *Glomus*, two of *Acaulospora* and one each of *Sclerocystis, Scutelospora Entrophospora* and *Gigaspora* were recorded in all. *Rauwolfia serpentina* showed maximum species richness (11 species) followed by *Coleus aromaticus* (10), *Adathoda vasica* (9) and *Andrographis peniculata* (7). *Glomus aggregatum, G.macrocarpum, G. fasciculatum, G. mosseae, Acaulospora appendiculata* and *Scutellospora* sp. were found in all effective colonizing species where as *Scerocystis* sp. was found only in the rhizosphere of *Rauwolfia serpentina* and *Entrophospora* sp. was found with *Coleus aromaticus* and *Adathoda vasica* only.

The comparative data of destribution, frequency and density of AMF spores in effective colonizing species is depicted in Table -3. The data revealed that *Glomus aggregatum, G. macrocarpum, G. fasciculatum, G. mosseae, Acaulospora appendiculata* and *Scutellospora* sp. were found dominating with 100% frequency, where as *Acaulospora laevis* and *Gigaspora* sp. came in moderate frequency range (75%) followed by lower ranges of *Glomus constrictum, Glomus geosporum, Entrophospora* sp. (50%) and *Sclerocystis* sp. (25%). *G. fasciculatum, G.mosseae* and *Acaulospora appendiculata* showed maximum densities (3.0) among the recorded AMF species. Genus *Glomus* emerged as a dominant member among all. The present results are in agreement with the findings of Sharma *et al.* (2007a) that the species richness and spore density of VAM fungi depends upon the area and agro-climatic conditions; however, they reported genus *Acaulospora* as a predominant genus in the medicinally important plants of Himachal Pradesh.

Table 1 : AM fungal Association and Spore Population with cultivated medicinal plants.

S. No.	Name of plant species	Botanical Name	% Colonization in root	Structure Seen			Spore Population/100g Soil
				H	V	A	
1.	Akarkara	*Spilanthus acmella* Murr.	20	++	+	+	65 ± 1.27
2.	Pipli	*Piper longum* L	36	+	–	–	25 ± 1.22
3.	Vasaka	*Adathoda vasica* L	60	+++	+	–	110 ± 1.34
4.	Patharchur	*Coleus aromaticus* L	52	+++	–	+	187 ± 1.45
5.	Sarpgandha	*Rauwolfia serpentina* L	72	+++	+	+	260 ± 1.21
6.	Mint	*Mentha piperita* L	18	+	–	–	72 ± 1.28
7.	Chitrak	*Plumbago compansis* L	28	+	+	+	86 ± 1.11
8.	Kalmegh	*Andrographis peniculata* L	47	++	+	+	97 ± 1.37
9.	Giloe	*Tinospora cordifolia* L	20	+	–	–	45 ± 1.25
10.	Bach	*Acorus calamus* L	42	++	–	+	78 ± 1.28
11.	Brahmi	*Becopa monnieri* L	12	+	–	–	40 ± 1.12
12.	Ashvagandha	*Withania somnifera* L	25	+	+	–	56 ± 1.36

H-Hyphae and Hyphal structures; V- Vesicles; A- Arbuscles,
+ Present; – Absent
+++ High profusely branched hyphae within cortex
++ Moderate hyphae

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Table 2 : Predomination of AM Fungal spores in the effective colonizing plant species.

S. No.	Name of Effective Colonizing Plant Species	Predominated AMF Spores (Species Richness)						No.of AMF Species Found
		Glomus sp.	*Acaul.*sp.	*Giga.*sp.	*Scut.*sp.	*Scle.*sp.	*Entro.*sp.	
1.	*Rauwolfia serpentina*	Ga,Gc, Gf,Gm, Gma,Gg	Aa, Al	Giga	Scut	Scle	-	11
2.	*Coleus aromaticus*	Ga,Gc, Gf,Gm Gma	Aa, Al	Giga	Scut	-	En	10
3.	*Adathoda vasica*	Ga,Gf, Gm,Gg Gma	Aa, Al	-	Scut	-	En	09
4.	*Andrographis peniculata*	Ga,Gf Gm Gma	Aa	Giga	Scut	-	-	07

Ga-*Glomus aggregatum*; Gma-*Glomus macrocarpum*; Gf-*Glomus fasciculatum*; Gm-*Glomus mosseae*; Gg-*Glomus geosporum*; Gc-*Glomus costricum*; Giga-*Gigaspora* sp.; Aa-*Acaulospora appendiculota*; Al-*Acaulospora laevis*; Scle-*Sclerocystis* sp.; Scut-*Scutellospora* sp.; En-*Entrophosphora* sp.

Table 3 : Distribution, diversity, frequency and density of AM fungal spores in the effective colonizing species.

S. No.	Name of AMF Species	No. of AMF spores				Total	%F	D
		A	B	C	D			
1.	Ga	3	2	1	2	8	100	1.6
2.	Gma	2	3	4	3	12	100	2.4
3.	Gf	4	6	3	2	15	100	3.0
4.	Gm	2	4	6	3	15	100	3.0
5.	Gc	3	2	–	–	5	50	1.0
6.	Gg	4	–	2	–	6	50	1.2
7.	Giga	2	3	–	4	9	75	1.8
8.	Aa	4	2	4	5	15	100	3.0
9.	Al	2	2	3	–	7	75	1.4
10.	Scle	1	–	–	–	1	25	0.2
11.	Scut	3	1	1	1	6	100	1.2
12.	En	–	2	1	–	3	50	0.6

A. *Rauwolfia serpentina* B. *Coleus aromaticus* C. *Adathoda vasica* D. *Andrographis peniculata*

It can be concluded that this study provides preliminary data on the status of indigenous AM fungi in cultivated medicinal plants, which will help to demonstrate their impact on crop growth and to develop best supportive combination for their cultivation studies.

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Allelopathic Potential of Rice Plant Parts on Germination and Seedling Growth of *Echinochloa colona*.

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The use of rice (*Oryza sativa* L.) allelopathy for weed control is a new technology in agriculture. The plant part (Leaves, stem, root) extracts from 12 distinctly different plant type cultivated rice genotypes were used to determine their allelopathic potential on seed germination and seedling growth of *Echinochloa colona*. In this study, the extract of rice plant parts were significantly reduced the germination percentage and seedling growth parameters particularly shoot length, root length, seedling dry weight and vigour index of *Echinochloa colona*. Genotypes Vasumati, Dubraj and Safri-17 plant parts showed maximum adverse impact than other genotypes. These results suggest that rice plant parts may be a source of natural herbicide and there may be genetic variation among rice genotypes for allelopathic potential on *Echinochloa colona*.

Keywords : Allelopathy, germination percentage, vigour index, seedling dry weight.

INTRODUCTION

Weeds are a constant constraint to rice production and cause yield losses in all rice production systems and in all seasons (Zoschke, 1990). In weeds *Echinochloa* spp. is one of the worst weeds in irrigated rice systems, causing large yield losses every season. It was estimated in Malaysia that the yield loss by grasses (mainly *Echinochloa* spp.), broad leaved weeds and sedges was 41, 28 and 10 per cent, respectively (Azmi, 1992). *Echinochloa colona* was selected as a test species for this study because it is one of the most trouble some weeds in rice production all over the world. Allelopathy refer to the detrimental effects of higher plants of one species (the donor) on the germination, growth or development of plants of another species (the recipient). It is different from the competition because the detrimental effect is exerted through release of chemical inhibitors (allelochemicals) by the donor species to the plant environment. Allelochemicals virtually present in all plant parts and allelopathy depends on the quantity of allelochemicals present in leaves, stem and roots which constitute major portion of plant. Therefore, an attempt was made extracts from 12 cultivated rice genotypes on *Echinochloa colona* germination and seedling growth parameters.

MATERIAL AND METHODS

Twelve rice genotypes were grown at the instructional farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur during *kharif* 2006 and 2007. Individual plants were tagged on the basis of name of genotypes. Individual plants and their parts (leaves, stem/ shoot and root) were collected and required parts were separated carefully for further process. The contaminants like soil and other foreign material were removed by washing gently with tap water for few seconds. Take sample 5 g (leaves/ shoot/ stem and root) were soaked in 100 ml distilled water for 24 hours at room temperature. Then solution were filtered through cheese cloth to remove debris and then centrifuged at 3000 rpm for 30 minutes. The supernatant was filtered through Whatmann filter paper No. 1 and prevent microorganism growth. First of all, in each sterilized 9 cm petri dish, Whatmann Filter paper No 1 was filled. After this, trash was removed from the seeds and 100 healthy seed of *Echinochloa colona* were placed on filter paper. Thereafter, 10 ml of aqueous extract of different parts of rice and control (D.W.) was added to each petri dish as per treatments. Then, per cent germination was determined after 5 days, and seedling root and shoot length were measured after 9 days. Seedlings were separated and oven dried at 65ºC for 4 hours and measured dry weight. Per cent inhibition was calculated as:

$$\text{Per cent inhibition} = \frac{\text{Control - Aqueous extract}}{\text{Control}} \times 100$$

Vigour index was computed as suggested by Abdul Baki and Anderson (1973) using following formula:

Vigour index = Seedling length x Germination percentage

The experiment was conducted in a completely randomized design with three replications. The treatments were allotted to different Petri dishes by using random method (Gomez and Gomez, 1984).

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RESULTS AND DISCUSSION

Seed germination : The leaf, stem and root extract of rice genotypes significantly reduced the germination percentage of *Echinochloa colona* as compared to control. In the leaf extract most adverse impact was observed in Vasumati and least adverse impact was observed in R-1182-167-2-157-1 (Table 1). In stem extract minimum germination observed in Vasumati. But ,maximum recorded in R-1060-1674-1-1 (Table 2). In root extract also maximum adverse impact was observed in Vasumati and minimum adverse impact note in Indira Sugandhit dhan (Table 3). The inhibition of *Echinochloa colona* germination may reflect the allelopathic potential of individual rice cultivars. Results of this study are in agreement with those of Chung *et al.* (2003), Olofsdotter *et al.* (2002) and Dilday *et al.* (1994), who concluded that variation in allelopathic activity existed among cultivars.

Seedling growth parameters : The plant part extracts of rice genotypes significantly reduced the seedling length of *Echinochloa colona* as compared to control. The leaf extracts from Vasumati and R-1072-360 were the most and least inhibitory of *Echinochloa colona* shoot and root length respectively (Table 1). Almost similar effect of these genotypes was observed in stem and root extract (Table 2 & 3). Similar finding given by Kim *et al.* (2005) and recorded that water extract of shoots and roots significantly inhibited root growth in *Echinochloa crusgalli* but the shoot extract gave a greater inhibitory effect on *Echinochloa crusgalli* than the root extract.

The plant part (leaf, stem and root) extracts of rice genotypes significantly reduced the seedling dry weight *Echinochloa colona* as compared to control. Maximum adverse impact was observed in rice genotype Vasumati. Whereas, minimum adverse impact was observed in leaf, stem and root extracts of rice genotypes R-1060-1674-1-1, R-1182-167-2-157-1 and R-979-1528-2-1 respectively (Table 1, 2 & 3).

The plant part extracts of rice genotypes significantly reduced the vigour index of *E. colona* as compared to control. The minimum vigour index recorded in Vasumati but maximum vigour index observed in leaf, stem and root extracts of rice genotype R-1072-360, R-1060-1674-1-1 and Indira Sugandhit dhan respectively (Table 1, 2 & 3).

The leaf extract from Vasumati and R-1060-1674-1-1 were the most (40.55%) and least (23.8%) inhibition of seedling dry weight, respectively (Table 1). In the stem extract maximum inhibition recorded in Vasumati (42.95%) and minimum inhibition observed in R-1182-167-2-157-1 (25.4%) (Table 2). In root extract maximum adverse impact recorded in Vasumati (34.84%) and minimum per cent inhibition observed in R-1182-167-2-157-1 (25.25%) (Table 3). Some investigators have suggested that measuring seedling length is complicated due to curling and other morphological alteration, seedling dry weight may be the better selection criteria for allelopathic potential Chung *et al.*, 2003 and reveled that cultivar 'Jaganbyeo' reduced *E. colona* seedling dry weight up to 96%.

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Table 1 : Effect of rice leaf extract on *Echinochloa colona* germination and growth parameters.

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling dry weight (g)	Vigour index	Seedling dry weight (g) per cent inhibition
R-1037-649-1-1	75.0	8.0	6.7	0.830	1102.2	28.75
Danteshwari	74.0	7.9	6.6	0.821	1072.9	29.52
R-979-1528-2-1	77.0	8.3	7.0	0.823	1178.3	28.35
Vasumati	65.0	6.6	4.5	0.652	721.6	40.55
R-1182-167-2-157-1	77.5	7.9	7.1	0.851	1162.4	25.50
R-1072-360	76.0	8.9	7.5	0.820	1246.0	28.35
Indira Sugandhit Dhan	75.5	8.3	6.8	0.832	1140.2	28.80
R-548-89-6	76.5	8.1	7.0	0.841	1158.9	27.20
Safri-17	71.0	6.9	5.0	0.703	844.6	36.45
Dubraj	67.0	6.7	4.7	0.651	763.8	39.30
R-1249-1196-2-1	77.0	7.9	6.5	0.853	1108.8	25.20
R-1060-1674-1-1	75.5	7.7	7.1	0.872	1117.1	23.80
Control	81.0	10.1	9.0	1.170	1528.0	-
SEm±	**0.960**	**0.171**	**0.144**	**0.011**	**18.73**	**2.013**
CD at 5%	**2.935**	**0.524**	**0.444**	**0.035**	**57.53**	**6.204**

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Table 2 : Effect of rice stem extract on *Echinochloa colona* germination and growth parameters.

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling dry weight (g)	Vigour index	Seedling dry weight (g) per cent inhibition
R-1037-649-1-1	77.0	8.4	7.4	0.840	1220.6	26.85
Danteshwari	75.5	8.3	7.3	0.849	1181.5	25.90
R-979-1528-2-1	77.5	8.4	7.6	0.841	1240.0	26.45
Vasumati	73.0	7.7	6.6	0.652	1047.7	42.95
R-1182-167-2-157-1	77.5	8.5	7.4	0.858	1236.2	25.40
R-1072-360	78.5	8.5	7.7	0.818	1271.6	29.05
Indira Sugandhit Dhan	78.5	8.6	7.5	0.842	1267.8	27.60
R-548-89-6	77.0	8.4	7.5	0.822	1224.3	28.50
Safri-17	75.5	8.2	6.8	0.731	1136.2	36.45
Dubraj	74.0	7.9	6.7	0.670	1084.0	41.25
R-1249-1196-2-1	77.5	8.5	7.4	0.801	1232.3	30.70
R-1060-1674-1-1	79.0	8.5	7.6	0.820	1275.7	28.35
Control	83.0	9.8	8.5	1.151	1523.0	-
SEm±	**0.720**	**0.112**	**0.090**	**0.0042**	**15.60**	**0.563**
CD at 5%	**2.201**	**0.344**	**0.277**	**0.0129**	**47.69**	**1.735**

Table 3 : Effect of rice root extract on *Echinochloa colona* germination and growth parameters.

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling dry weight (g)	Vigour index	Seedling dry weight (g) per cent inhibition
R-1037-649-1-1	74.5	7.9	7.2	0.824	1132.4	25.28
Danteshwari	75.0	8.0	7.1	0.819	1132.5	26.35
R-979-1528-2-1	73.5	8.1	7.4	0.838	1142.8	25.30
Vasumati	64.5	7.5	6.2	0.646	883.8	34.85
R-1182-167-2-157-1	75.0	8.1	7.0	0.818	1132.5	25.25
R-1072-360	73.0	8.1	7.2	0.810	1117.0	27.15
Indira Sugandhit Dhan	76.5	8.2	6.9	0.821	1158.9	26.55
R-548-89-6	75.5	8.0	7.1	0.814	1143.8	27.05
Safri-17	70.0	7.7	6.6	0.723	1001.2	36.00
Dubraj	66.0	7.6	6.4	0.664	927.3	30.25
R-1249-1196-2-1	75.0	8.1	7.0	0.822	1132.3	26.00
R-1060-1674-1-1	75.0	8.1	7.2	0.821	1147.8	26.55
Control	79.0	9.7	8.4	1.112	1433.8	-
SEm±	**0.843**	**0.083**	**0.099**	**0.0320**	**16.56**	**0.679**
CD at 5%	**2.577**	**0.254**	**0.302**	**0.0099**	**50.61**	**2.094**

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Impact of Biofertilizers on the Growth and Yield of Moong (*Vigna radiata* L.)

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An experiment was conducted during summer season of 2009 at Sultnapur, district Kannauj (U.P.) to study the impact of biofertilizers on the growth and yield of moong. Two levels of chemical fertilizers (60 & 80 Kg N ha^{-1}) with the combination of different biofertilizers (PSB, *Azotobacter, Azospirillum*) were used. The difference in the growth parameters such as plant height, length to branch, no. of pods, pod length and graia yield was studied at the interval of 35 and 70 days. Plant treated with PSB+80 Kg N ha^{-1} produced the highest yield.

INTRODUCTION

Green gram (*Vigna radiata* L.) is native to India where it has been cultivated since ancient times. It is widely cultivated in Maharashtra, Uttar Pradesh, Gujarat, Tamil Nadu, Andhra Pradesh and Bihar, occupying an area of about 2.4 Million hectares with a production of 1 million tones of seeds. The crop responds well to NPK fertilizers.

It has been reported that continuous use of inorganic sources of nutrients has led to deterioration of soil health and resulting in stagnation or reduction in productivity of crops. (Ahmad *et al.*, 2008). Biofertilizers are products containing living cells of different types of micro–organisms that have the ability to mobilise nutrients from unusable form through biological processes and these groups of micro–organisms may either fix atmospheric nitrogen or solubilise insoluble phosphorous and make them available for crops. *Azospirillum*, an associative microphilic nitrogen fixer, is commonly found in association with the roots of cereals. It is capable of fixing over 30 Kg N ha^{-1}. Normally 2.0 Kg of *Azospirillum* is required per ha (Swedrzynska *et al.*, 2001). Phosphorus solubilising bacteria (PSB) are known to have the ability to solubilise and transfer inorganic phosphorus from normally insoluble sources through excretion of various organic acids and other means. It has also been reported that phosphorus uptake efficiency and yield increases with inoculation of phosphorus bacteria (Gyaneshwar *et al.*, 2002). They can be applied and recommend for all crops. *Azotobacter* is a non–symbiotic nitrogen fixing bacteria which performs well if the soil organic matter content is high. *Azotobacter* is capable of fixing up to 30 Kg N ha^{-1} yr^{-1}.

Karthikeyan *et al.* (2008) highlighted the effect of interspecific competition between natural fertilizers (cow dung), chemical fertilizers (urea) and biofertilizers (Phormidium tenue, Bradyrhizobium sp.) on the over all growth performance and reproductive yield of moong. In general, increase in yield due to biofertilizer application was of the order of 15-20%. On the basis of current results and those discussed above, a study was conducted to asses the impact of biofertilizers on the growth and yield of moong.

MATERIAL AND METHODS

Field experiment was conducted during summer season of 2009 at Sultnapur, District Kannauj (U.P.) in RBD (Random block design) with three replications. The experimental plots of 1m^2 were prepared for sowing moong variety *i.e.*, PM–4 obtained from IARI, New Delhi.

The treatment combinations were as follows:–

T_1	–	Control
T_2	–	*Azospirillum* (*Azs*) + 60 Kg N ha^{-1}
T_3	–	Phosphorus solubilising bacteria (PSB) + 60 Kg N ha^{-1}
T_4	–	*Azotobacter* (*Azt*) + 60 Kg N ha^{-1}
T_5	–	PSB + 80 Kg N ha^{-1}
T_6	–	*Azs* + 80 Kg N ha^{-1}
T_7	–	*Azt* + 80 Kg N ha^{-1}
T_8	–	PSB + *Azs* + *Azt* + 60 Kg N ha^{-1}
T_9	–	PSB + *Azs* + *Azt* + 80 Kg N ha^{-1}

Biofertilizers were applied through seed inoculation just prior to sowing. Urea was used after Ist irrigation as a source of N. Moong was sown during April 2009 using seed rate of 20 Kg ha^{-1}. Row to row distance was kept at 30 cm and plant to plant distance was 10 cm with the depth of sowing 4-5 cm. Crop matured in 75 days.

Data regarding plant height, no. of branch plant^{-1}, no. of pods, pod length and grain yield (Kg ha^{-1}) was recorded.

RESULTS AND DISCUSSION

The maximum plant height was recorded in T_5 *i.e.*, treatment PSB + 80 Kg N ha^{-1}. The minimum plant height was observed in T_1 *i.e.*, control. The maximum no. of branch was observed in T_3 and T_5 *i.e.*, treatment PSB+60 Kg N ha^{-1} and PSB+80 Kg N ha^{-1}. However, the maximum number of pods plant^{-1} was recorded in T_5. The minimum no. of pod plant^{-1} was observed in T_1. The increased pod formation in treatment where PSB+80 Kg N ha^{-1} were applied may be attributed due

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Table 1 : Effect of different biofertilizers on the growth and yield of moong (70 DAS).

Treatment	Plant height (cm)	No. of branch plant^{-1}	No. of pod plant^{-1}	Pod length (cm)	Grain yield Kg ha^{-1}
T_1	23.45	6.66	40	3.45	1100
T_2	29.33	7.33	64.66	3.91	1400
T_3	30.9	**8.66**	70.16	4.86	2200
T_4	31.13	7.33	71.16	4.31	1250
T_5	**32.88**	**8.66**	**76.16**	**5.15**	**2300**
T_6	31.06	8.00	57.16	4.28	1500
T_7	29.61	8.16	65.16	4.3	1600
T_8	32.3	6.83	69.5	4.95	1800
T_9	30.71	8.00	60.83	4.66	1750

to better plant development through efficient utilization of PSB by the plant. Similarly the maximum pod length was observed in T_5. Phosphorus improved the number of pods, pod length and seed weight. These results were in close comformity with those of Naeem *et al.*, (2006). Among the treated groups the highest yield was recorded in T_5. The minimum grain yield was noted in T_1 (Table-1). Biofertilizers are used in biological control of soil–borne diseases and stimulate to great extent, plant growth by producing growth regulators. (Sheateawi and Tawfik, 2007).

It has been recorded that biofertilizers increased the number of pods plant^{-1} up to 20.1% over control (Karthikeyan, *et al.*, 2008). Moreover, foliar spray with cyanobacteria (Phormidium faveoloarum) recorded 41.76% increase in number of pods plant^{-1}, 48.78% increase in weight of pods plant^{-1}, 64.7% increase in number of seeds plant^{-1} & 60.21% increase in weight seeds plant^{-1} over untreated control. (Shanthi, *et al.*, 2004). According to Srinivas and Shaik (2008) the decrease in number of grains pod^{-1} may attributed to de'iciency of essential nutrients (NPK) at the time of flowering and seed setting stage of plant. Because at this stage phosphorus is required by the plant to complete such important growth stages like that of flowering and seed setting optimally, while K is required by the plant to improve the seed quality.

The increase in yield attributing characters and yield in T_5 treatment may be due to the fact that phosphorus plays vital role in root development and proliferation and also improves nodule formation and nitrogen fixation by supplying assimilates to the roots, energy transformation and metabolic process in plants, resulting in to greater photosynthesis and synthesis of protein and carbohydrates. Hence T_5 treatment (PSB+80%N ha^{-1}) show the maximum yield. These results were confirmed earlier by Joseph and Verma (1994) and Sarkar, *et al.*, (1995).

On the basis of above cited discussion, it can be documented that the combination of PSB+80% N ha^{-1} (T_5) is better for moong crop. Findings of this study provided a sound base to believe that biofertilizers increased the growth and yield of moong crop and proved to be beneficial for obtaining the maximum amount.

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Nitrate and Nitrite Reductase Enzyme Activity in Bean, Okra, Guar and Onion Seedlings as Influenced by Organo-phosphorus Pesticides

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The effect of Methylparathion and Phosphamidon on the activity of nitrate and nitrite reductase in the seedlings of bean, okra, guar and onion was studied. Nitrate reductase activity in the Phosphamidon treated guar seedlings was 4 to 7 fold more. On the contrary, Methylparathion treatments were prominently inhibitory. In general a stimulatory effect of the activity of nitrate reductase observed in bean and okra. However there was no effect of the insecticides on onion seeds.

Keywords : Bean, Guar, Methylparathion, Nitrate reductase, Nitrite reductase, Okra, Onion, Phosphamidon.

INTRODUCTION

Vegetable crops like bean, okra, guar and onion are susceptible to the pest attack. To control these pests, organophosphorus pesticides *viz.*, Methylparathion and Phosphamidon are used in large scale. The indiscriminate use of these pesticides leads to environmental pollution and the adverse effects as produced also the subject of growing concern today (Tatekar *et al.,* 1977). Although there are several reports on the effects of pesticides on the seedling growth but biochemical studies regarding pesticide mediated changes in germinating seeds are relatively scanty (Guha *et al.,* 1987). Therefore, it is alarmingly important to screen the seeds or plants for biochemical effects of almost all the chemicals being used commercially. In the present investigation, therefore an attempt has been made to study the effect of Methylparathion and Phosphamidon on the activity of enzyme nitrate reductase and nitrite reductase in seedlings of bean, okra, guar and onion.

MATERIAL AND METHODS

Seeds of kidney bean (*Phaseolus vulgaris* L.), okra (*Abelmoschus esculentus* Moench L.), guar (*Cyamopsis tetragonoloba* L.) and onion (*Allium cepa* L.) were first surface sterilized, with 0.1% $HgCl_2$ for 1 min followed by repeated washing with distilled water and then were allowed to germinate in dark, in the incubator at 30°C in corning glass petridishes lined with filter paper, each having 10 ml aqueous solution of Methylparathion : 0.01, 0.02, 0.03 and 0.04% (all v/v) and Phosphamidon 0.005, 0.01, 0.015, 0.02% (v/v). Simultaneously controls (distilled water) were maintained under identical conditiions. The enzyme activities were studied after 72h of germination. Activities of the enzyme nitrate reductase (E.C. 1.6.6.1) and nitrite reductase (E.C. 1.6.6.4) were determined by the method of Jaworsky (1971).

RESULTS AND DISCUSSION

From the present investigation it is clear that, the nitrate reductase activity in bean is stimulated with increasing concentration of Methylparathion but higher concentrations exhibitied inhibitory effect (Table-1). On the contrary, only lower and higher concentrations of Phosphamidon showed stimulatory effect. In okra, the nitrate reductase activity was comparatively less affected. In case of onion, this enzyme showed a negative correlation with the increasing concentration of both the pesticides.

The nitrite reductase activity in bean, okra and onion has increased with increasing concentration of both Methylparathion and Phosphamidon with an exception of okra and onion where it was considerably inhibited at 0.02% Phosphamidon (Table-2). The nitrite reductase activity in guar was slightly inhibited at lower concentrations of both the pesticides. However, at higher concentrations it was more inhibited by Phosphamidon as compared to that by Methylparathion.

Sairam *et al.* (1988) studied effect of herbicide - Atrazine and 2, 4 - D on nitrate reductase activity in *Sorghum* and recorded increase in nitrate reductase activity was highest at 30 days after sowing followed by a decline at 53 and 70 days. Maximum increase in the activity of this enzyme was recorded for 2, 4 - D application followed by Atrazine and combined application of 2, 4 - D and Atrazine.

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Table 1 : Effect of Methylparathion and Phosphamidon on the activity of enzyme nitrate reductase* in the seeds of bean, okra, guar and onion (72 h germination).

Treatments		Nitrate reductase (µg NO_2 liberated h^{-1} mg^{-1} protein)			
		Bean	Okra	Guar	Onion
	Control	4.95	0.24	0.42	0.21
Methylparathion % (v/v)	0.01	8.36	0.14	0.088	0.21
	0.02	10.08	0.48	0.101	0.20
	0.03	6.98	0.25	0.107	0.19
	0.04	1.49	0.13	0.065	0.18
Phosphamidon % (v/v)	0.005	7.27	0.23	1.62	0.12
	0.010	2.74	0.24	2.73	0.19
	0.015	3.34	0.20	1.89	0.11
	0.020	7.28	0.21	2.33	0.08

*Values are mean of three determinations.

Table 2 : Effect of Methylparathion and Phosphamidon on the activity of enzyme nitrite reductase* in the seeds of bean, okra, guar and onion (72 h germination).

Treatments		Nitrite reductase (µg NO_2 reduced h^{-1} mg^{-1} protein)			
		Bean	Okra	Guar	Onion
	Control	3.30	0.12	0.44	0.035
Methylparathion % (v/v)	0.01	9.30	0.13	0.35	0.11
	0.02	18.26	0.13	0.41	0.09
	0.03	20.27	0.15	0.41	0.11
	0.04	6.68	0.11	0.38	0.12
Phosphamidon % (v/v)	0.005	5.58	0.12	0.43	0.10
	0.010	6.89	0.13	0.45	0.09
	0.015	8.00	0.14	0.42	0.09
	0.020	3.50	0.08	0.12	0.05

*Values are mean of three determinations.

The decreased nitrate reductase activity was noticed by Churchill and Klepper (1979) in Ametryn herbicide treated wheat seedlings. Shanmugasundaram *et al.*, (1996) have investigated the effect of Dimethoate, Quinolophos and Cypermethrin on nitrate reductase activity in four species of marine angiosperms and found that Dimethoate inhibited nitrate reductase activity in all four species of plants studied and Cypermethrin showed stimulatory effect in only one plant *Halophila ovata*. It is interesting to note that, present findings are also in similar lines. Singh *et al.*, (1998) observed a decrease in nitrite reductase activity in 2, 4-D treated cowpea seedlings. A tremendous increase was observed in nitrate reductase activity. They concluded that, 2, 4-D selectively affects the pathway of nitrate reductase. The same may be reson for increased reductase activity recorded in present study.

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Boron Application on Uptake of Boron in Tomato (PKM 1)

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A field experiment was carried out at Agricultural College and Research Institute, Madurai to evaluate the application of boron (B) in tomato for content and uptake of B in shoot at various growth stages and fruit. Application of B significantly increased the uptake of B. Application of 20 Kg borax ha^{-1} recorded the highest content and uptake of B in vegetative, flowering and harvest stage and in fruit. Among the foliar sprays, 0.25 per cent borax spray at 50 and 90 days after planting registered the highest B uptake in both shoot and fruit of tomato. The results also revealed that soil application of B had a more pronounced effect in increasing the uptake of B as compared to foliar sprays and control.

Keywords : B application, uptake, shoot, fruit, tomato (PKM 1).

INTRODUCTION

Tomato is an important vegetable crop rich in mineral, protein and vitamin. It plays a vital role in Indian economy by virtue of its various modes of consumption in human diet. Micronutrients are very much essential for the growth and development of plants. Micronutrients are required by plants in very small quantities, yet they are very effective in regulating plant growth due to enzymatic action. Different crops require varied quantities of micronutrients in their different stages of growth (Shrotriya and Phillips, 2002). Multiple cropping with high yielding varieties of crop is one of the important causes of removal of micronutrients from the soil. Under deficiency of micronutrients, the growth of plant is hampered; the plants are subjected to attack by disease and the yield of the crops decreases accordingly (Das, 2008).

Boron is one of the micronutrient; the primary function of B in plant is to maintain cell wall structural integrity. Boron provides cross links between cell wall polysaccharides that gives structure to cell wall - important for cell expansion, regulation of H^+ transport, retention of cellular Ca^{2+} and control of lignin production following cell expansion (Parr and Loughmann, 1983). Cell wall structure is important for normal transport of water, nutrients and organic compounds required for new growth. Boron is essential for pollen tube growth and seed development (Havlin *et al.,* 2006). Under B deficiency, normal cell wall expansion is disrupted. Fruit cracking is specific symptom of B deficiency which affects the economic value of tomato. The B nutrition of vegetable crops has greater impact in Tamil Nadu due to large scale cultivation of tomato in vegetable growing areas covering an extent of 22924 ha with a total production of 3 lakh tonnes of tomato per annum (Anon., 2009). Hence, to evaluate the application B on uptake of B the present research work was undertaken in B deficient soils.

MATERIAL AND METHODS

A field experiment was undertaken during the year 2006 in modern orchard of Agricultural College and Research Institute, Madurai with tomato (variety PKM 1) as the test crop in B deficient soils of Madukkur soil series. The experimental soil (Madukkur soil series) was sandy loam in texture and is taxonomically grouped under Typic Haplustalf. The available nitrogen was low (185 Kg ha^{-1}), medium in available phosphorus (18.05 Kg ha^{-1}) and medium in available potassium (185 Kg ha^{-1}). The soil was neutral in reaction (pH 7.5). The available B (0.36 ppm) was found to be lower than critical value of 0.5 ppm and the total B was 27.5 ppm

The experiment consisted of 12 treatments including control and was laid out in randomized block design (RBD) replicated thrice. The net plot size was 2.0 m x 4.0 m. Treatments schedule is given in Table 1. Recommended package of practices were adopted for raising the crop. Shoot samples were collected at three stages *viz.*, vegetative (30 days after planting), flowering stage (60 days after planting) and harvesting stage (90 days after planting). Fruit samples were collected at harvest stage of crop. The collected samples were dried in shade and then in hot air oven at 60°C and ground in a Wiley mill using stainless steel grinder and stored in labelled containers for chemical analysis. The B content was estimated by adopting standard procedure (Piper, 1966). The uptake was calculated by multiplying the percentage of B with dry matter production and expressed in g ha^{-1}. The data obtained from the present investigation were subjected to

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statistical analysis is as described by Panse and Sukhatme (1967).

Table 1. Treatment structure

T_1 - Control
T_2 - 5 Kg borax ha^{-1}
T_3 - 10 Kg borax ha^{-1}
T_4 - 15 Kg borax ha^{-1}
T_5 - 20 Kg borax ha^{-1}
T_6 - 25 Kg borax ha^{-1}
T_7 - 0.25 % borax spray twice at flowering stage (50th and 80th day)
T_8 - 0.50 % borax spray twice at flowering stage (50th and 80th day)
T_9 - 0.25 % borax spray twice at fruit initiation stage (60th and 90th day)
T_{10} - 0.50 % borax spray twice at fruit initiation stage (60th and 90th day)
T_{11} - 0.25 % borax spray twice at flowering and fruit initiation stage (50th and 90th day)
T_{12} - 0.50 % borax spray twice at flowering and fruit initiation stage (50th and 90th day)

All plots were fertilized N, P_2O_5 and K_2O @ 120: 60: 50 Kg ha^{-1}

RESULTS AND DISCUSSION

B content and uptake in shoot and fruit : The mean value of B content of tomato shoot at various growth stages was found to be significant (Table 2).

The B content of shoot increased from vegetative (30.60 ppm) to flowering stage (36.40 ppm) and declined thereafter in harvest (33.49). This might be due to the translocation of metabolites to reproductive phase (Singh and Srivastava, 1988). Regarding treatments, the highest value was recorded in soil application of borax @ 20 Kg ha^{-1} (T_5). With regards to foliar application, the highest value was recorded in 0.25 per cent borax spray on 50 and 90 days after planting (T_{11}). The results also reported that soil application of B increased the B content of shoot as compared to foliar treatments. The increased B concentration in shoot with increasing levels of B application is primarily due to increased availability of B in soil. Similar results were also reported by Singh and Dixit (1994) in cauliflower, Prabha (1995) in tomato and Annie Varghese (2003) in cauliflower. The simple correlation relating mean available B content of soil with B content in shoot was positively correlated with each other (r = 0.890**) (Table 4).

The B content in fruit ranged from 10.3 to 14.7 ppm. Application of borax @ 20 Kg ha^{-1} (T_5) recorded the highest value and the lowest value was in control. The B content in fruit increased with increasing levels of soil application of borax as compared to foliar treatments (Table 2). The availability of B in soil increases by the addition of borax which enhanced the absorption of B in fruit. Similar results were also reported by Rahmatullah *et al.* (1999), Yadav and Manchanda (1982) in Mustard. The mean available B content of soil was significantly correlated with B content in fruit (r = 0.870**) (Table 4).

The B uptake in shoot was found to be significant at various growth stages of tomato. The highest value was recorded in flowering stage whereas the lowest value in vegetative stage. The results also revealed that the uptake of B increased from vegetative to flowering stage and decreased at harvest. This might be due to the increased content in shoot, which resulted in higher uptake in flowering stage. Sakal *et al.* (2002) reported that mobilization of B from source to sink decreased the concentration in later stages of crop growth. Soil and foliar application of B also significantly influenced the uptake of B by shoot. The highest value was recorded in treatment borax @ 20 Kg ha^{-1} (T_5). The results also revealed that soil application of B increased the uptake of B in shcot as compared to foliar treatments and control (Table 3). This might be due to increased concentration in soil and enhanced uptake of B. The uptake of nutrients by the crop, mainly governed by the biomass production combined with higher content of the element in those treatments might be the reason for higher uptake values (Prabu, 2000). Several authors have also reported increased B uptake due to the B application (Singh *et al.*, 1994 and Singh and Dixit, 1994). Increased uptake of B by various treatments reflected the higher requirement of B by the crop (Gupta and Cutcliffe 1972). A significant positive correlation was observed between mean available B content of soil and B uptake in shoot (r = 0.92**) (Table 4).

The results also revealed that soil application of B had a significant influence on higher B uptake in fruit as compared to the rest of the treatments (Table 3). Boron helped to increase the fruit weight, number of fruits and also increased the B uptake of fruit that contributed to the yield (Singh and Verma, 1991). The mean available B content and B uptake in fruit was found to be significantly correlated with each other (r = 0.83**) (Table 4).

Total B uptake of tomato ranged from 511.3 to 1151.50 g / ha (Table 3). The highest value was recorded in treatment borax @ 20 Kg ha^{-1} (T_5) and was found to be significantly superior to rest of the treatments. This might be due to the increased concentration in soil which enhanced the uptake of B in shoot and fruit. Several authors have also reported that the increase in B uptake due to B application (Singh and Dixit, 1994). Among the various levels of foliar treatments the highest value was recorded in 0.25 per cent borax spray twice on 50 and 90 days after planting (T_{11}). The results also revealed that soil application of borax showed a higher total uptake as compared to rest of foliar treatments and could be attributed to increase in uptake in shoot and fruit which ultimately resulted in higher total uptake of B. A significant positive correlation was observed between mean available content with total uptake of PKM 1 tomato (r = 0.89**) (Table 4).

Boron application had a profound effect in increasing the availability of B in soil which inturn resulted in higher uptake of B by tomato. In recapitulation, it can be clearly stated that soil application of borax @ 20 Kg ha^{-1} (T_5) had a significant effect on B content and its uptake of tomato (PKM 1) in boron deficient soils of Madukkur soil series.

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Table 2 : B application on B content of shoot (at various growth stages) and in fruit of tomato.

Treatments	Shoot			Mean	Fruit
	Vegetative (S_1)	Flowering (S_2)	Harvest (S_3)		
T_1	16.80	18.00	17.70	17.50	10.3
T_2	29.20	33.56	30.88	31.21	13.2
T_3	29.50	34.20	31.46	31.72	13.3
T_4	29.60	35.72	32.86	32.72	13.7
T_5	30.60	36.40	33.49	33.49	14.7
T_6	30.00	36.01	33.13	33.04	14.1
T_7	17.80	28.78	26.48	24.35	10.8
T_8	17.90	30.09	27.68	25.22	12.1
T_9	17.80	29.16	26.83	24.59	11.1
T_{10}	17.80	29.97	27.57	25.11	11.7
T_{11}	17.90	32.40	29.81	26.70	12.9
T_{12}	17.90	31.70	29.16	26.25	12.4
Mean	22.81	31.33	28.85		

Shoot	SEd	CD (P=0.05)	Fruit	
S	0.07	0.14	SEd	0.05
T	0.14	0.29	CD (P=0.05)	0.11
S x T	0.25	0.51		

Table 3 : B application on B uptake of shoot (at various growth stages), in fruit and total uptake (g / ha) in tomato.

Treatments (T)	Shoot			Mean	Fruit	Total
	Vegetative (S_1)	Flowering (S_2)	Harvest (S_3)			
T_1	139.2	149.2	146.7	145.0	364.6	511.3
T_2	294.0	337.9	310.9	314.3	652.0	963.0
T_3	304.1	352.6	324.3	327.0	666.3	990.7
T_4	314.9	380.0	349.6	348.2	694.5	1044.2
T_5	336.2	400.0	368.0	368.1	783.5	1151.5
T_6	325.5	390.7	359.4	358.5	740.2	1099.7
T_7	155.2	250.9	230.9	212.3	429.8	660.7
T_8	167.1	281.0	258.5	235.5	516.6	775.2
T_9	162.5	266.2	244.9	224.5	449.5	694.5
T_{10}	164.2	276.6	254.4	231.7	489.0	743.5
T_{11}	179.1	324.3	298.4	267.3	606.3	904.7
T_{12}	171.1	303.0	278.7	250.9	539.4	818.1
Mean	226.7	309.4	284.8			

Shoot	SEd	CD (P=0.05)		Fruit	Total
S	0.92	1.83	SEd	5.41	7.94
T	1.84	3.67	CD (P=0.05)	11.22	16.48
S x T	3.19	6.36			

Table 4 : Correlation coefficient between available B with different dependent variables.

Available B content	B content in shoot	0.890**
Available B content	B content in fruit	0.870**
Available B content	B uptake in shoot	0.920**
Available B content	B uptake in fruit	0.830**
Available B content	Total B uptake	0.890**

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Evaluation for Drought Tolerance in Elite Genotypes of Bread Wheat (*Triticum aestivum* L.)

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Twenty eight diverse wheat genotype were evaluated during two *Rabi* seasons of 2005–06 and 2006–07 under two environmental conditions *viz.*, Irrigated (E_1) and moisture stress (E_2). As there has not been significant increase in the wheat production in rainfed areas of the country in general and state in particular. About 86 million hectares of rainfed area in India and around 70 per cent area falls under wheat in Jammu and Kashmir state is rainfed with no dependable source of irrigation. Little attempt has been made to breed specifically for drought resistance in wheat largely due to lack of unique and easily quantifiable plant attributes. The basic requirements of improvements of drought resistance are availability of its rapid and reliable method for screening large number of breeding materials and its adequate genetic diversity responsive to drought stress. It is a complex physiological and morphological character involving enormous number of genes (Boyer 1996). No other environmental factor limits global crop productivity more severely than water deficit (Boyer 1982). The grain yield was positively correlated with days to maturity, number of grains per ear, number of tillers per plant and plant height. Similarly grain yield was negatively correlated with days to maturity, number of grains per ear, number of tillers per plant and plant height. Similarly grain yield was negatively correlated with 1000 grain weight harvest index, whileas grain yield was positively correlated with days to fifty per cent flowering, days to maturity, plant height, straw weight, spike weight, number of tillers per plant, number of grains per ear and ear length.

Keywords : Wheat, Drought Tolerance, Genetic Variability, Correlation.

INTRODUCTION

Globally, drought causes more yield losses than any other factor. Every year the yield is lost due to insufficient soil moisture. As wheat area expands on less suitable lands, and as the effects of climate change are increasingly felt, it is likely that more wheat crops experience drought, particularly in wheat growing areas. Therefore development of varieties with increased drought tolerance and water use efficiency is crucial to improving the productivity and sustainability of the wheat crop. For identifying superior types, these are designated to develop screening techniques based on morphological and physiological traits associated with performance under water limited conditions.

Drought–Making best of water resources through breeding. Globally drought causes more yield losses than any other factor (Boyer 1982). Drought losses are on average 1-2t/ha. (Foulkes *et al.*, 2007). Further more climate change models predict that summer time droughts will worsen (Jones *et al.*, 2003, Richter and Seminov, 2005). In arid regions improvements in drought tolerance have been made largely through conventional breeding that is selecting varieties that yield well in target dry environments. Though there is substantial genetic variability for drought tolerance in wheat (Foulkes *et al.*, 2001). Breeders find it difficult and costly to breed effectively for these traits and drought tolerance is defined on the basis of yield. Marker assisted selection (MAS) is an attractive option, but this requires the identification of Key loci for yield performance under drought. Qualitative traits loci for drought tolerance per se have so far shown limited utility.

Wheat (*Triticum aestivum* L.) is a staple food of above 40 per cent human population across globe. In India it occupies second place, in wheat for last eight years and maintained its position in the world next to only China and at present produces more wheat than the United States of America. Total world consumption is around 595 million tonnes per year and this is expected to grow over the coming years. Wheat products such as bread, biscuit, pasta and noodles regularly appear in the shopping lists. The number of consumes of these products is increasing with change in life style. India has been giving special emphasis to improve productivity of wheat under harsh environments *viz.*, drought and high temperature. The problems in rainfed area are having no means to irrigate such undulated lands, and being totally dependent on weather. Secondly no suitable variety could be developed so that it could surpass/ sustain the vagaries of climate for establishing the crop of wheat

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in these so there is dire need of development of drought tolerant varieties and secondly we have to investigate the methods to identify drought resistant donors.

MATERIAL AND METHODS

The experiment was laid ant at the experiment farm of SKUAST–J, Chatha Jammu, during the *Rabi* season of 2005-06 and 2006-07, to evaluate these cultivars of wheat, these entries were tested in the Randomized Block Design with three replications and each of these treatments constituted of two lines with a row spacing of 22.5 cm apart and of 1.50 meters length. The seed material collected was tested in two environments *viz.*, (E_1) and (E_2) for two years. Consequently *viz.*, in controlled conditions in, earthen pots and in irrigated conditions in the field. The characters studied are variability studies and secondly the methods used were correlation with yield in dry condition and irrigated conditions for two years 2005-06 & 2006-07 and data presentad in Table-1. Wheat being a promising cereal crop possess second rank after rice in India. Wheat production was subsequently increased with the introduction of dwarf Mexican wheat from 1967, but production decreased from 1996–97. Hence there was an immediate attention for grain yield improvement of yield through selection depends largely on the amount of genetic variability like yield is not very effective. Therefore, in present investigation knowledge of correlation among the complex characters and their effects on to estimate the amount of genetic advance for yield and its components.

Higher heritability of spike yield (337.20) followed by straw yield (131.79) and days to fifty per cent flowering (178) followed by plant height (90.60), grains per ear (79.57) and this indicates the predominance of additive gene action and thus selection for these characters would be effective. However characters *viz.*, tillers per plant and days to maturity showed low heritability of the expression. These traits are mainly governed by several environmental and agronomic characters.

Major changes in plant was found in case of spike weight were the heritability was higher (326.71), while as genetic advance was (3737.205), being the highest, due to the favourable environment rather than genotypic effect (Singh *et al.*, 1997) incase of grains per ear, plant height, the heritability was of medium order but genetic advance for traits was (1179.6) and (2010.96) respectively. What being a promising cereals crop possesses second rank after rice in India. Wheat production was substantially increased with the introduction of drear Mexican wheat from 1967, but production decreased from 1996–97, hence there is an immediate attention for grain yield improvement. Genetic improvement of yield through selection depends largely on the amount of genetic variability present in the population.

Heritability and genetic advance under selection for a complex character like yield is not very effective. Knowledge of correlation among the complex characters and their effects on these characters is carried out to estimate the amount of genetic variability, heritability and genetic advance for yield and its components, Correlations among these characters and their analysis with seed yield in wheat cultivars.

RESULTS AND DISCUSSION

Estimates of variability, heritability and genetic advance for yield and its components characters are presented in (Table-2). Among the characters studied, days to 50 per cent flowering, plant height, ear length, number of grains per ear, 1000 grain weight and seed yield exhibited with range of variability. Phenotypic and Genotypic coefficient of variations for number of tillers, number of ears per plant, plant height, 1000 grain weight were compared to other characters namely harvest index. For straw yield, number of grains per ear and grain yield. The components of genotypic and phenotypic variances were high. Suggesting effectiveness of the phenotypic and genotypic selection (Paroda and Joshi, 1970) reported that number of ears per plant, grains per ear and grain weight were the major yield components. Higher heritability of (326.71) per cent was recorded for spike yield, number of tillers per plant, grains per ear and days to maturity. It was negatively correlated for plant height, Harvest index, 1000 grain weight, days to fifty per cent flowering. The Phenotypic correlation with number of tillers and days, to 50 per cent flowering was negatively correlated with grain yield, 1000 grain weight, and harvest index. The plant height was positively correlated, however low positively correlation of days

Table 1 : Different plant characters showing range, mean, CV, GCV, PCV, heritability and genetic advance.

Character	Range	Mean/SE	C.V.%	G.C.V.	P.C.V.	h_2b heritability	Genetic advance	GA%mean
Days to 50% flowering	95–102	0.599	0.36	0.38	0.286	178.397	304.521	105.28
No. of Tillers/plant	8–27	3.49	0.023	17.286	33.76	26.2106	269.34	1823
Plant height	89-128	14.812	17.04	9.663	10.181	90.066	2010.965	1889.07
Straw yield	11–32	3.7049	0.1178	0.0938	-0.0665	215.4344	1133.837	29.446
Spike wt.	3–27	3.011	6.3504	11.4786	6.3504	326.71	3737.2	6435.53
Days to maturity	128-143	2.3141	0.6932	0.1926	0.7194	7.1656	43.42	10.62
Grains/ear	15-29	2.6552	13.796	27.2248	30.5208	79.5789	1179.16	50.0028
Grain yield/plant	3–90	7.8623	15.953	22.2327	27.3646	0.66	22.458	37.4259
1000gr. wt.	33.52	0.9896	1.059	23.05	21.58	106.81	1022.14	737.1

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Table 2 : Estimate of coefficients of variance variability, heritability and genetic advance for various characters in wheat.

Character		Variance		Heritability (%)	Genetic Advances	Genetic advance as % of mean	Coefficient of variance	
		Genotypic	Phenotypic				Genotypic	Phenotypic
		2	3	4	5	6	7	8
Days to 50% of flowering	Y_1/E_1	1.42	1.74	0.82	2.23	2.32	1.25	1.38
	Y_1/E_2	256.60	276.13	0.93	31.81	33.36	16.80	17.43
	Y_2/E_1	10.47	44.78	0.23	3.17	7.36	3.39	7.02
	Y_2/E_2	14.26	60.64	0.30	4.01	2.10	3.96	7.21
No. of days to maturity	Y_1/E_1	8.19	14.29	0.57	4.44	3.26	2.09	2.77
	Y_1/E_2	13.56	22.99	0.59	5.82	4.26	2.70	3.51
	Y_2/E_1	0.62	8.65	0.07	3.12	2.72	0.58	2.15
	Y_2/E_2	10.82	18.53	0.58	5.18	3.79	2.41	3.15
Plant height (cm)	Y_1/E_1	20.83	25.79	0.81	4.22	3.85	4.17	4.64
	Y_1/E_2	26.51	31.69	0.84	9.70	0.64	4.71	5.15
	Y_2/E_1	23.05	24.52	0.94	9.59	8.76	4.53	4.38
	Y_2/E_2	253.27	272.81	0.93	31.57	3.26	14.55	15.10
No. of tillers/Plants	Y_1/E_1	3.39	3.84	0.88	3.56	263.70	136.38	145.15
	Y_1/E_2	2.51	3.38	0.74	2.81	208.14	117.35	136.18
	Y_2/E_1	6.52	24.88	0.26	2.69	199.25	189.14	369.48
	Y_2/E_2	0.83	2.02	0.41	58.39	4325.18	67.48	105.28
Ear length (cm)	Y_1/E_1	0.58	2.69	0.22	0.72	7.48	7.92	17.05
	Y_1/E_2	0.67	4.43	0.15	0.64	6.65	8.51	21.88
	Y_2/E_1	0.84	1.99	0.42	1.23	13.30	9.53	14.66
	Y_2/E_2	2.08	4.29	0.94	0.78	8.11	14.99	21.54
Number of grains/ear	Y_1/E_1	14.29	20.91	0.68	6.44	71.31	41.86	50.64
	Y_1/E_2	10.02	13.13	0.76	5.70	63.12	35.05	40.13
	Y_2/E_1	41.18	51.75	0.79	11.79	130.56	79.66	79.66
	Y_2/E_2	27.03	28.19	0.96	10.49	116.16	58.57	58.79
Biomass/Plant (g)	Y_1/E_1	53.05	60.32	0.88	14.08	1223.47	633.35	675.36
	Y_1/E_2	23.75	28.89	0.82	9.08	791.30	423.77	467.39
	Y_2/E_1	8.42	15.52	0.54	4.38	382.60	252.32	342.57
	Y_2/E_2	10.68	18.79	0.57	5.09	441.73	284.18	376.93
Grain yield/plant (g)	Y_1/E_1	2.69	2.86	0.94	3.27	7.02	3.41	3.62
	Y_1/E_2	3.49	3.51	0.99	3.82	8.22	4.00	4.01
	Y_2/E_1	188.71	272.79	0.69	3.30	7.07	29.43	35.39
	Y_2/E_2	6.75	6.76	0.99	5.30	11.46	5.57	5.57
Harvest index	Y_1/E_1	6.66	10.71	0.62	4.18	10.87	6.69	8.40
	Y_1/E_2	1.64	2.8	0.58	0.60	1.55	3.32	4.34
	Y_2/E_1	215.09	220.06	0.97	29.64	77.54	38.07	38.51
	Y_2/E_2	14.78	16.89	0.87	7.36	19.23	9.98	10.67
1000 Grain weight (g)	Y_1/E_1	3.06	24.11	0.13	1.13	2.70	3.69	10.36
	Y_1/E_2	2.01	16.15	0.12	1.03	2.17	2.99	8.48
	Y_2/E_1	23.05	24.52	0.94	9.59	20.24	10.13	10.45
	Y_2/E_2	21.79	23.29	0.93	7.41	19.63	9.85	10.19

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Relative water content of leaf Y_1/E_1	2.53	2.80	0.90	1.28	5.84	13.58	17.71
Y_1/E_2	3.33	3.44	0.97	1.03	6.63	11.95	14.59
Y_2/E_1	1.34	1.45	0.92	9.59	4.12	11.99	12.28
Y_2/E_2	2.34	2.45	0.96	9.30	24.23	11.99	12.28
Relative water loss of leaf Y_1/E_1	3.00	5.10	0.59	3.26	21.53	13.58	17.71
Y_1/E_2	2.32	3.46	0.67	2.57	20.16	11.97	14.59
Y_2/E_1	2.34	2.45	0.96	3.09	24.24	11.99	12.28
Y_2/E_2	1.39	2.45	0.55	0.52	13.88	9.11	12.92
Proline Content Y_1/E_1	0.07	0.19	0.37	0.30	6.19	0.05	0.08
Y_2/E_2	0.01	0.06	0.17	0.11	1.50	1.87	4.59

Table 3 : Correlation between grain yield and various drought related traits.

Character	Correlation
Drought susceptibility Index Vs Yield (S)	0.5809**
Drought susceptibility Index Vs Yield (NS)	0.3727**
Germination Stress Index Vs Yield (S)	0.1132**
Germination Stress Index Vs Yield (NS)	0.0525*
Relative water content Vs Yield (S)	0.1806*
Relative water content Vs Yield (NS)	0.114*
Relative Water Loss Vs Yield (S)	–0.1759**
Relative Water Loss Vs Yield (NS)	–0.1020**
Proline Vs Yield (S)	–0.0280*
Proline Vs Yield (NS)	0.082*
Seed Died Vs Yield (S)	0.1182**
Seed Died Vs Yield (NS)	–0.153**

**,* Significant at 5 and 1 per cent level, respectively.

to maturity, number of tillers per plant, grains per ear (Table 3). These results are in agreement with those of (Singh *et al.*, 1979). However days to maturity v/s days to 50 per cent flowering negatively correlated and was significant, 1000 grain weight, ear length, spike weight, grain yield, grains per ear, harvest index were positively correlated with days to 50 per cent flowering. Plant height with spike weight, straw weight but had negative association with harvest index, 1000 grain weight, ear length and grain yield, thus further suggested the initiation of increasing straw yield without reduction of harvest index.

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Evaluation of Greengram Genotypes for Morphological Traits, Dry Matter Accumulation and Partitioning

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A field experiment was conducted during *Kharif* season under rainfed condition with 30 greengram genotypes belonging to varying yield potential. Significant variations were observed among the genotypes with respect to the morphological traits, dry matter accumulation and its partitioning pattern and yield. Among the morphological traits, number of primary branches, number of green trifoliate and dry weight of root nodules were found significantly higher in high yielding genotypes. The high yielding genotypes also possessed higher leaf dry weight, stem dry weight and total dry weight as compared to medium yielding, low yielding genotypes and checks. Among the genotypes, M-446 and M-100 had higher seed yield and other yield components. The same genotypes were also found more efficient in dry matter accumulation and its partitioning and may be used as genetic source for improvement of yield potentials in greengram.

Keywords : Genotypes, Dry Matter partitioning, Morphological traits, Seed yield.

INTRODUCTION

Greengram is the third most important pulse crop in India. Like any other grain legumes its national average productivity is very low, *i.e.,* 380 Kg ha^{-1}. (Anon., 2002). The lower productivity of greengram can be attributed mainly to poor physiological efficiency of the crop, *viz.*, poor partitioning of assimilates to the economic sink, abscission of floral parts, canopy architecture, poor pod setting, etc. (Alberda and Bower, 1983). In order to break the yield barrier and to improve the yield, there is an urgent need to develop an ideal plant type with determinate and compact growth habit, higher harvest index coupled with optimum dry matter production per unit area and its efficient partitioning to the economic sinks (Jain,1975). The crop yield depends on the accumulation of photo assimilates during the growing period and the way in which they are partitioned between the desired organs of the plants (Bhattacharya, 1996). At present it is felt necessary to identify the morphophysiological parameters, dry matter accumulation and its partitioning pattern which govern the productivity and also to screen those genotypes on the basis of these characters. However, the research reports in this regard are scanty. Therefore, the present investigation was conducted with an aim to evaluate 30 greengram genotypes for morphophysiological parameters, dry matter accumulation and its partitioning in relation to seed yield.

MATERIAL AND METHODS

A field experiment was conducted during *Kharif* season with 30 greengram genotypes belonging to varying yield potentials under rainfed condition on vertisols of University of Agricultural Sciences, Dharwad, Karnataka. The genotypes were classified into three groups on the basis of yield performance, *viz.*, high yielding (seed yield is more than 6.5 g/ plant), medium yielding (seed yield is in between 4.4 to 6.5 g/ plant) and low yielding (seed yield is less than 4.4 g/plant). The available nitrogen (%), phosphorus (P_2O_5 Kg / ha.) and potassium (K_2O Kg / ha.) in the soil of experimental plot were 0.05, 40.00 and 320.00, respectively. The total rainfall received during the crop growing season was 214.2 mm. The experiment was laid out in a randomized block design with three replications during *kharif*, 1999 and the seeds were sown in 15th. June. The crops were grown following the recommended package of practices and proper plant protection measures were also adopted at right time.

Ten plants were tagged randomly in each plot avoiding boarder rows for recording morphological parameters, yield attributes and seed yield. Plant height was measured from the ground level to the growing tip. The number of leaves, the number of primary branches and nodes on the main stem were counted from the bottom to the tip ot the plant. Five plants were randomly selected from each net plot area and uprooted carefully without destroying the root system at 45 days after

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sowing (45 DAS). The roots were washed in running water and the nodules were counted. These nodules were separated and dried in an oven at 80°C temperature for 72 hours to record the dry weight.

The days required from sowing to 50% flowering and physiological maturity was recorded in each genotype. Five plants were sampled randomly at 15, 30, 45 days after sowing and at harvest from each plot and separated into leaves, stems and reproductive parts (flowers and pods), dried separately in a hot air oven until a constant weight was attained and recorded the dry weight of each part separately. Total dry weight was calculated by adding the dry weight of all the parts. Seed yield and yield components were recorded from the ten tagged plants which were used for recording the morphological parameters. Shelling per cent was calculated by dividing the seed yield by pod yield, while harvest index (HI) was worked out by dividing the seed yield by total dry weight and expressed in percentage. The data were subjected to the analysis of variance following the standard statistical procedure.

RESULTS AND DISCUSSION

Significant genotypic differences were observed with respect to morpho-phenological parameters (Table 1). Among the genotypes, M–42 recorded significantly higher plant height and higher number of nodes on the main stem. On the other hand significantly lower plant height was registered by Pusa baisakhi followed by the genotype TARM-18. The number of primary branches at harvest ranged from 1.0 to 2.5 among the genotypes. Among the genotypes, M-446 and Co. 2-1 possessed higher number of primary branches followed by M-100, whereas Colco LM-5 recorded the minimum number of primary branches per plant. The genotypes belonging to high yielding group maintained significantly higher number of primary branches per plant as compared to the genotypes of medium yielding and low yielding groups as well as checks.

A progressive increase of the number of leaves per plant was observed with an advancement of crop growth from 15 to 45 DAS, thereafter declined up to harvest irrespective of the genotypes. The genotypes belonging to high yielding group maintained significantly higher number of leaves per plant as compared to the genotypes belonging to medium yielding and low yielding groups as well as checks. At 45 DAS and harvest, M-446 had significantly higher number of leaves followed by M-100. The genotypes 64 and 54 were found to possess significantly lower number of leaves at 45 DAS and harvest, respectively. The number of root nodules among the genotypes ranged from 16.0 to 28.3. Among the genotypes, LM-590 had significantly higher number of root nodules, while the minimum number was recorded by the genotype M-42. Among the genotypes, the dry weight of root nodules ranged from 0.11 to 0.26 g. per plant. The genotype M-446 maintained significantly higher nodule dry weight followed by M-100. The genotype LM-532 and 20 recorded the minimum nodule dry weight. Days to 50% flowering and maturity ranged from 37.3 to 48.3 days and 62.0 to 73.0 days, respectively among the genotypes. Among the genotypes, 54 needed significantly higher and Colco. LM-5 required lower number of days for 50% flowering. Among the genotypes, LM-672 registered significantly higher and the genotype 20 needed lower number of days for physiological maturity.

A wide genotypic variability with respect to plant height, number of nodes, primary branches and leaves were reported by several researchers in greengram (Yohe and Poehlman, 1972; Naidu *et al.*, 1993; Sahu and Patra, 1997 and Dodwad, 1997). Evans (1975) reported that plant height and the number of branches were influenced by the interaction between the environmental variables and genetic make-up of the plant. Several workers reported that the number of branches in grain legumes may be considered as one of the important factors influencing seed yield and also found a significant positive association between the number of primary branches and seed yield in greengram (Malik *et al.,* 1987; Chandrababu, 1990 and Borah and Hazarika, 1995). The results of the present investigation are in agreement with these reports. Leaf is an important parameter in determination of dry matter production and yield in greengram (Kuo *et al.,* 1977). Chandrababu (1990) also reported a significant positive association of leaf number with seed yield in greengram. The results of the present investigation also clearly indicate the importance of leaf number in determination of productivity in greengram. Presence of root nodules in pulses helps the crop in fixing atmospheric nitrogen and hence there exists a positive association between the nodule dry weight and seed yield in pulses. Paul (1998) reported that rhizobial inoculation increased the nodule dry weight and seed yield in greengram. The results of the present investigation also suggest that dry weight of nodules is an important factor for achieving higher productivity in greengram. The time of flowering and maturity are important factors affecting crop productivity and adaptation to diverse agro–climatic situation. Several workers have reported a high genotypic variability with respect to days to 50 per cent flowering and maturity among greengram genotypes (Naidu *et al.,* 1993; Sahu and Patra, 1997 and Dodwad, 1997), which are in agreement with the results of the present in investigation.

Significant differences were observed among the genotypes with respect to leaf dry weight, stem dry weight and total dry weight per plant at all the stages of crop growth (Table 2). There was a progressive increase of stem dry weight and total dry weight per plant with an advancement of crop growth from 30 DAS up to harvest irrespective of the genotypes. Leaf dry weight increased progressively from 30 to 45 DAS and decreased thereafter up to the harvest. The genotypes belonging to high yielding group maintained significantly higher leaf dry weight, stem dry weight and total dry weight as compared to the genotypes belonging to medium yielding and low yielding groups as well as checks. At 30 DAS, M-100 possessed significantly higher leaf dry weight followed by M-446, Pusa-9071 and M-1, while M-123 registered significantly lower leaf dry weitht. At 45 DAS, M-100 recorded significantly higher leaf dry weight followed by M-1, Co. 2-1, Pusa-9071 and M-446, whereas, LM 5-12 recorded significantly lower leaf dry weight. At harvest, M-446 and LM 5-12 recorded higher and lower leaf dry weight, respectively. The genotypes M-446 was found to possess significantly higher stem dry weight at 30 DAS and at harvest, whereas Varsha recorded higher stem

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Table 1 : Morpho-phenological parameters in greengram genotypes.

Genotypes	Plant height at harv.	Node/ plant harv.	Branches /plant harv.	Leaves /plant 45 DAS	Leaves/ plant at harvest	Nodules plant at 45 DAS	Nodule dry wt. (g/ pl)	50% flowering (days)	Maturity (days)
High yielding genotypes									
M–446	49.9	9.8	2.5	17.7	12.3	21.6	0.26	40.7	66.7
M–100	58.7	10.2	2.4	17.0	12.0	23.0	0.24	40.5	65.0
Co. 2–1	51.9	9.3	2.5	15.3	9.0	23.5	0.19	44.3	69.3
M–1	62.3	10.3	2.1	15.0	10.0	18.8	0.19	41.0	65.3
Pusa–9071	47.8	9.3	2.0	15.0	9.3	25.4	0.23	42.3	68.7
TARM–18	40.7	8.0	2.0	14.3	10.0	20.1	0.20	37.7	68.3
Mean	51.9	9.5	2.3	15.7	10.4	22.1	0.22	41.1	67.2
Medium yielding genotypes									
ML–3	53.7	9.1	1.6	16.0	8.3	20.3	0.16	44.0	69.7
42	51.7	8.8	2.2	15.0	8.0	23.0	0.15	42.3	67.0
LM–54	50.4	9.3	2.0	16.2	10.0	25.4	0.20	40.0	63.7
ML–4	55.8	9.1	2.0	15.0	8.3	20.0	0.15	45.0	69.0
LM–532	46.9	9.1	2.0	15.0	9.0	16.8	0.10	45.0	64.3
97	51.7	9.1	1.6	14.0	8.0	22.0	0.14	42.3	64.0
LM–272	42.7	8.4	1.3	13.3	8.0	19.0	0.15	41.0	62.7
LM–421	46.5	9.4	1.4	13.0	8.0	20.2	0.18	40.7	64.0
Varsha	56.3	9.4	1.3	13.0	8.3	16.4	0.11	45.7	65.3
LM–672	50.4	8.9	1.3	14.3	8.0	16.7	0.14	46.3	73.0
LM–590	46.1	9.1	2.0	12.7	7.7	28.3	0.13	44.0	68.7
M–42	68.4	12.3	1.3	14.0	8.0	16.0	0.17	39.7	65.0
Mean	51.7	9.3	1.7	14.3	8.3	20.3	0.15	43.0	66.4
Low yielding genotypes									
64	46.7	9.0	1.5	11.3	6.7	18.0	0.13	45.3	66.3
Anjugan	50.4	9.1	1.3	14.0	8.0	21.1	0.12	44.0	65.7
237	60.4	10.3	1.3	14.0	7.0	19.5	0.14	41.0	69.3
LM–87	57.2	10.5	1.2	14.0	9.3	17.3	0.14	47.7	67.3
20	42.7	8.4	1.2	15.2	7.7	18.8	0.10	40.7	62.0
Colco LM–5	42.4	8.2	1.0	12.0	7.0	19.0	0.11	37.3	67.3
LM–641	57.3	10.4	1.3	12.0	7.0	18.7	0.12	45.3	69.3
54	40.7	8.2	1.2	13.3	5.7	20.1	0.14	48.3	68.3
LM 5–12	52.4	9.4	1.5	12.2	7.7	17.9	0.13	46.0	67.7
M–123	49.4	9.4	2.0	13.3	7.0	19.0	0.11	37.7	63.0
Mean	50.0	9.3	1.3	13.1	7.3	18.9	0.12	43.3	66.6
Check									
Pusa baisakhi	35.7	8.1	1.1	15.0	8.0	20.5	0.14	39.7	64.7
China mung	43.7	8.2	1.3	14.0	8.3	22.1	0.15	41.7	65.3
Mean	39.7	8.2	1.2	14.5	8.2	21.3	0.15	40.7	65.0
Grand mean	50.4	9.3	1.6	14.2	8.4	20.3	0.15	42.6	64.5
CD (5%)	4.6	2.0	0.5	2.6	1.2	4.5	0.03	5.4	6.0

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Table 2 : Dry matter partitioning in leaf and stem and total dry weight in greengram genotypes at different stages of growth.

Genotypes	Leaf dry wt. (g plant^{-1})			Stem dry wt. (g plant^{-1})			Total dry wt. (g plant^{-1})		
	30 DAS	45 DAS	Harvest	30 DAS	45 DAS	Harvest	30 DAS	45 DAS	Harvest
High yielding genotypes									
M–446	1.06	5.60	3.23	2.13	4.17	6.62	3.19	11.03	27.39
M–100	1.10	6.43	3.09	1.94	3.55	6.46	3.04	10.95	24.05
Co. 2–1	1.00	5.91	3.21	1.91	3.42	6.13	2.91	9.23	21.61
M–1	1.03	6.28	3.02	1.83	2.78	6.51	2.86	10.12	21.97
Pusa–9071	1.05	5.73	3.10	1.91	3.34	6.27	2.96	9.87	21.53
TARM–18	0.98	5.40	3.08	1.83	2.43	5.78	2.81	9.58	20.32
Mean	1.04	5.89	3.12	1.93	3.28	6.30	2.96	9.96	22.81
Medium yielding genotypes									
ML–3	0.96	5.04	2.99	1.39	2.41	6.13	2.35	8.24	19.06
42	1.00	5.19	2.73	1.12	2.41	6.32	2.12	8.25	19.65
LM–54	0.92	3.12	2.00	1.09	3.20	5.43	2.01	7.65	18.21
ML–4	1.00	5.10	3.15	0.97	2.41	6.11	1.97	8.35	19.03
LM–532	0.98	4.81	2.68	1.47	2.73	5.48	2.45	8.45	19.79
97	1.02	3.28	2.06	1.47	3.21	5.38	2.49	6.89	16.66
LM–272	0.95	3.00	1.88	1.79	3.57	5.11	2.74	9.02	16.22
LM–421	0.90	2.83	1.74	1.06	2.93	4.88	1.96	7.12	16.18
Varsha	0.96	3.02	1.81	1.55	4.28	4.98	2.51	7.91	16.66
LM–672	0.95	3.82	2.00	2.06	4.20	5.92	3.01	9.02	18.52
LM–590	1.00	4.01	2.01	1.22	3.30	5.82	2.22	7.86	18.01
M–42	0.93	3.02	1.71	1.92	3.21	5.29	2.85	7.33	16.00
Mean	0.96	3.85	2.23	1.43	3.16	5.57	2.39	8.01	17.83
Low yielding genotypes									
64	1.00	3.04	1.55	1.67	3.10	5.53	2.67	7.52	16.51
Anjugan	0.91	3.43	1.52	1.30	3.80	6.16	2.21	8.56	17.01
237	0.95	2.92	1.78	1.23	3.66	5.30	2.18	7.36	16.35
LM–87	0.90	3.03	1.71	1.08	3.32	5.84	1.98	7.03	16.00
20	0.90	4.02	2.06	1.46	3.30	6.38	2.36	8.33	16.35
Colco LM–5	0.86	2.48	1.30	0.92	3.34	5.22	1.78	6.91	14.50
LM–641	0.92	3.00	1.51	1.09	3.91	5.29	2.01	7.96	15.17
54	0.85	3.01	1.24	0.71	3.04	5.18	1.56	6.83	13.69
LM 5–12	0.86	2.48	1.15	0.92	3.30	5.56	1.78	7.04	14.38
M–123	0.83	2.51	1.37	0.51	3.46	5.18	1.34	6.71	12.62
Mean	0.90	2.99	1.52	1.09	3.42	5.56	1.99	7.43	15.26
Check									
Pusa baisakhi	0.98	3.98	1.32	1.14	2.67	5.63	2.12	7.25	14.32
China mung	1.00	3.98	2.26	0.76	2.52	4.54	1.76	7.03	15.90
Mean	0.99	3.98	1.79	0.95	2.60	5.09	1.94	7.14	15.11
Grand mean	0.96	3.98	2.14	1.38	3.23	5.68	2.34	8.15	16.20
CD (5%)	0.03	0.70	0.56	0.44	0.49	0.92	0.61	1.98	2.33

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Table 3 : Yield components and seed yield in greengram genotypes.

Genotypes	Fruiting clus. /pl.	Pods/ Fruiting clus.	Pods/ pl.	Seeds/ pod	Pod length (cm)	100 seed wt. (g)	Shelling (%)	Harvest index (%)	Pod yield (g per plant)	Seed yield (g/pl)	Seed yield (Kg/ha)
High yielding genotypes											
M–446	7.8	5.1	40.0	14.3	8.7	3.53	62.31	39.31	17.54	10.93	1531
M–100	5.9	6.2	36.7	14.8	10.4	4.13	64.83	39.08	14.50	9.40	1468
Co. 2–1	6.5	5.0	32.3	14.0	7.7	2.60	59.76	33.92	12.27	7.33	1362
M–1	6.2	4.5	28.0	13.3	9.5	4.07	58.14	32.91	12.44	7.23	1300
Pusa–9071	6.3	5.0	31.5	13.3	7.6	2.67	58.94	33.30	12.16	7.17	1290
TARM–18	5.5	5.1	28.0	13.3	7.4	2.43	59.02	32.53	11.20	6.61	1201
Mean	6.4	5.2	32.8	13.9	8.6	3.24	60.50	35.27	13.35	8.11	1359
Medium yielding genotypes											
ML–3	5.1	5.0	25.5	14.3	7.5	2.87	57.75	30.90	10.20	5.89	1135
42	4.7	6.4	29.8	13.0	7.8	2.77	54.81	29.57	10.60	5.81	1054
LM–54	5.3	4.2	22.0	13.3	7.3	3.67	51.02	30.23	10.78	5.50	874
ML–4	5.3	5.0	26.3	13.0	7.7	2.73	53.91	27.69	9.77	5.27	946
LM–532	5.0	5.2	26.0	12.0	6.5	3.47	45.14	26.53	11.63	5.25	834
97	4.7	4.7	22.0	14.3	8.0	3.68	54.59	30.21	9.22	5.03	824
LM–272	4.5	4.3	19.5	15.3	8.7	3.50	53.41	30.41	9.23	4.93	803
LM–421	3.2	5.3	17.0	13.3	8.6	4.10	50.94	30.08	9.56	4.87	783
Varsha	4.3	4.3	18.3	13.0	7.4	3.00	47.32	28.63	9.87	4.77	780
LM–672	4.0	4.9	19.2	13.0	7.6	4.00	44.62	25.54	10.60	4.73	775
LM–590	3.4	6.5	22.0	12.2	7.8	3.53	46.21	26.09	10.17	4.70	764
M–42	3.4	5.3	18.3	13.0	7.3	2.93	48.11	27.50	9.00	4.43	744
Mean	4.4	5.1	22.2	13.3	7.7	3.35	50.65	28.58	10.05	5.09	860
Low yielding genotypes											
64	3.0	5.0	15.0	13.0	7.1	3.30	45.71	26.11	9.43	4.31	740
Anjugan	4.1	4.0	16.5	12.7	7.0	2.97	46.09	25.28	9.33	4.30	739
237	3.7	4.7	17.3	11.1	7.9	3.03	45.66	25.89	9.27	4.23	730
LM–87	3.7	4.9	28.0	12.0	7.0	2.53	48.85	25.83	8.56	4.13	700
20	4.1	3.6	15.0	11.3	6.1	3.23	42.98	20.80	7.91	3.40	590
Colco LM–5	3.6	4.6	16.5	13.0	7.8	2.83	42.50	23.45	8.00	3.40	581
LM–641	3.9	4.1	16.3	12.0	7.5	3.00	40.23	22.15	8.37	3.37	574
54	3.3	5.0	16.6	12.7	7.1	2.53	45.39	24.46	7.27	3.30	563
LM 5–12	3.7	4.6	17.0	11.1	6.8	2.97	42.11	22.48	7.67	3.23	501
M–123	3.2	4.4	14.0	13.0	7.1	3.10	41.68	20.07	6.07	2.53	317
Mean	3.6	4.5	16.2	12.2	7.2	2.95	44.12	23.65	8.18	3.62	604
Check											
Pusa baisakhi	4.1	3.9	16.0	12.0	7.3	3.20	46.54	23.97	7.37	3.43	602
China mung	4.7	4.5	21.0	13.3	7.6	4.00	49.45	28.30	9.10	4.50	753
Mean	4.4	4.2	18.5	12.7	7.5	3.60	48.00	26.14	8.24	3.97	678
Grand mean	4.5	4.8	22.1	13.2	7.7	3.21	50.54	28.11	9.97	5.13	862
CD (5%)	0.76	1.01	2.77	0.79	0.76	0.49	3.28	3.10	2.21	1.16	65.60

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dry weight at 45 DAS. The genotypes M-123, ML-3 and China mung possessed lower stem dry weight at 30 DAS, 45 DAS and at harvest, respectively, The genotypes M-446 and M-100 maintained significantly higher total dry weight while, M-123 had significantly lower total dry weight as compared to all other genotypes at all the stages of crop growth.

The amount of total dry matter (TDM) produced is an indication of the overall efficiency of utilization of resources and better light interception. Dry matter production and its partitioning, particularly to the reproductive organs is an important yield determining character in any crop. Therefore, the pattern of distribution of dry matter in different plant parts would give a better understanding of the genotypes. Kuo *et al.* (1977) and Ravi (1984) indicated that high yielding greengram genotypes were more efficient in the production of higher TDM and its efficient partitioning to the economic sinks. Similarly, several other workers also found a significant positive association of TDM with seed yield in greengram (Malik *et al.*, 1987; Dodwad *et al.*, 1998). The results of the present investigation are in agreement with these reports.

The data presented in Table 3 indicated significant genotypic variability with respect to yield components and seed yield in greengram. Among the genotypes, the seed yield ranged from 2.53 g. per plant (M-123) to 10.93 g. per plant (M-446), indicating clearly wide genotypes variability with respect to the parameter. Among the genotypes. M-446 registered significantly higher seed yield and yield components followed by M-100, Co, 2-1 M-1 and Pusa-9071. On the other hand, the genotype M-123 recorded significantly lower seed yield followed by LM 5-12, 54, LM-641, Colco LM-5 and 20. A wide genotypic variability with respect to seed yield and yield components among greengram genotypes were reported by several workers (Yohe and Poehlman, 1972; Jain, 1975; Borah and Hazarika, 1995 and Dodwad *et al.*, 1997). In general, crop yield depends on the accumulation of photo assimilates during the growing period and the way in which they are partitioned to the economic sinks. Again, the harvest index reveals the efficiency of translocation of assimilates towards economic parts and hence seed yield and harvest index are closely related, which has also been proved from the results of the present investigation. The primary yield components in greengram are the number of fruiting clusters per plant, pods per plant, seeds per plant, pod length, seed weight, etc. which are all quantitative characters and are genetically controlled. The genotypes M-446 and M-100 were identified as morpho–physiologically more efficient genotypes coupled with higher productivity and may be used as a genetic source for improvement of yield potential in greengram.

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A Biomathematical Approach to NaCl-Stress During Seed Germination and Seedling Growth of Ten Salt Tolerant Rice Cultivars

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Percentage of seed germination, young root and shoot length of 5-day-old seedlings of ten salt tolerant rice cultivars were studied under varying concentrations of NaCl-stress (0-400 mM). It was observed that the level of salt tolerance was cultivar - specific and stage-specific. The relative level of salt tolerance during seed germination, young root and shoot growth of these rice cutlivars has been presented in the form of a Venn diagram following the principle of set theory of mathematics. Cutlivars showing IC_{50} (50% inhibitory concentration) of more than 156 mM for seed germination and root growth and IC_{50} of more than 82mM for shoot growth were found to have high level of salt tolerance. IET 13559 was observed to be the most salt tolerant rice cultivar among the ten cultivars studied.

Keywords : NaCl-stress, Rice cultivars, Seed germination, Seedling growth.

INTRODUCTION

Among the abiotic stresses, salt stress is well known to adversely affect the growth and development of crop plants (Greenway and Munns 1980, Yeo and Flowers, 1986, Herralde *et al.* 1998, Gill and Sharma 1999, Grover *et al.* 1999, Sahu *et al.* 2001, Gobinathan *et al.* 2009). Germination of seeds and early and late seedling growth of different crop plants is known to be drastically affected by salt stress (Sheoran and Garg 1978, Greenway and Munns 1980, Alwan *et al.* 1989, Sahoo and Sahu 2003, Gobinathan *et al.* 2009). Rice (*Oryza sativa* L.) is an important crop of the globe. It is sensitive to salinity in the root environment, the grain yield of many varieties being reduced by half at 50 mol m^{-3} NaCl (Yeo and Flowers 1986). Rice (paddy) fields along the coastal areas of India have been drastically affected by salinity, mainly by NaCl. To reduce the salt level in these fields, at the present time, is economically unfeasible. It is, therefore, highly desirable that salt tolerant rice cultivars are grown in such areas. As a continuation of the screening procedure for the salt-tolerance, the present investigation was undertaken to study the germination and seedling growth of ten salt tolerant rice cultivars under varying concentrations of NaCl-stress to identify the most salt tolerant one and to present in a novel way the relative level of salt tolerance of these rice cultivars in the form of a Venn diagram following the principle of set theory of mathematics.

MATERIAL AND METHODS

Seeds of ten salt tolerant rice cultivars *viz.*, CR644, CRM 30, CSR 10, IET 12863, IET 13410, IET 13427, IET 13559, ORM 20-17-26, ORM 20-22-26 and ORM 26-25-35 were obtained from Saline Rice Research Station, Motto, Bhadrak, Orissa. These seeds were initially surface sterilized with 1% sodium hypochloride solution and were kept in warm water at 37°C for 1 hour. 20 healthy seeds were placed in Petri plate (dia-20cm) lined with filter paper moistened with 20 ml of distilled water (control) or different concentrations of NaCl (BDH) *viz.*, 50, 100, 150, 200, 300 and 400 mM and incubated in dark at 27° ± 2°C for a period of 5 days. Each treatment was replicated 5 times. Emergence of radicle by 2 mm was taken as the criterion for seed germination. For studying seedling growth, 10 germinated seedlings were taken at random at day 5 and root and shoot length were measured. The 50% inhibitory concentration (IC_{50}) of NaCl for seed germination, young root and shoot length for each cultivar was calculated by plotting the data in a graph (not shown). A Venn diagram was drawn to show the relative level of salt tolerance of these rice cultivars towards seed germination, root and shoot growth.

RESULTS AND DISCUSSION

In all the cultivars, the percentage of seed germination (Table 1), and shoot growth (Figure 1A, 1B and 2A, 2B) decreased on a NaCl concentration dependent manner. IC_{50} (50% inhibitory concentration of NaCl) at seed germination was lowest (105 mM NaCl) in CSR 10 and highest (205 mM) in IET 13559 (Table-2). However at young root and shoot growth stage, IC_{50} was found to be lowest (90 mM for root and 50mM for shoot) in case of IET 12863 and highest (210 mM for root and 125mM for shoot) in case of IET 13559 (Table 3). The degree of inhibition of seed germination by NaCl stress is in the following decreasing order :

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CSR10>IET 12863 > ORM 26-25-35 > CRM 30> IET 13410 > ORM 20-17-26 > ORM 20-22-26 > IET 13427 > CR 644 > IET 13559.

However, the above sequence of cultivars towards decreasing trend of inhibition by NaCl-stress was not found to be similar for young root and shoot growth stages, rather it was observed in the following sequence of decreasing order :

IET 12863 > CRM 30 > ORM 20-22-26 > IET 13427 > ORM 20-17-26 > ORM 26-25-35 > CSR 10 > CR 644 > IET 13410 > IET 13559.

From the above results it was found that IET 13559 is the most salt tolerant rice cultivar at all the three physiological parameters studied in this investigation.

The relative level of salt tolerance of ten rice cultivars during seed germination (A), young root growth (B) and young shoot growth (C) has been presented in a novel way in the form of a Venn diagram (Figure 3) by taking the data from Table 2 and 3. Cultivars showing IC_{50} of more than 156 mM for seed germination and root growth and IC_{50} of more than 82 mM for shoot growth were found to have high level of salt tolerance and less than the above values indicate comparatively low level of salt tolerance. The cultivars which are common in exhibiting same level of salt tolerance at all the three stages of growth are IET 12863, CRM 30, ORM 20-17-26, ORM 26-25-35, CR 644 and IET 13559.

In general, the inhibitory effect of NaCl-stress was found to be more pronounced on shoot growth than root growth and least on seed germination. These results are in conformity with findings of Sheoran and Garg (1978), Greenway and Munns (1980), Alwan *et al.* (1989) and Sahoo and Sahu (2003) that the level of salt tolerance is cultivar - specific and stage - specific.

In view of the above, it is concluded that IET 13559 is the most salt tolerant rice cultivar at germination and young

Table 1 : Percentage of seed gremination of ten rice cultivars under increasing concentration of NaCl. (Data at day 5 after incubation in dark at 27° ± 2°C).

Cultivar	Concentration NaCl in mM						
	0	50	100	150	200	300	400
CR 644	100	100	100	98	96	13	0
CRM 30	100	100	99	96	57	03	0
CSR 10	100	98	97	94	27	0	0
IET 12863	100	98	97	89	33	0	0
IET 13410	100	97	96	94	79	04	0
IET 13427	100	100	96	90	70	28	0
IET 13559	100	100	100	98	82	11	03
ORM 20-17-26	100	100	100	93	32	0	0
ORM 20-22-26	100	99	98	89	73	06	0
ORM 26-25-35	100	100	99	91	20	01	0

Table 2 : IC_{50} (in mM NaCl) for percentage of seed germination of ten rice cultivars in the increasing order of salt tolerance (Data at day 5 after incubation in dark at 27° ± 2°C).

Sl. No.	Cultivar	IC_{50}	Mean IC_{50}
1.	CSR 10	105	
2.	IET 12863	125	
3.	ORM 26-25-35	130	
4.	CRM 30	135	
5.	IET 13410	145	156
6.	ORM 20-17-26	150	
7.	ORM 20-22-26	180	
8.	IET 13427	185	
9.	CR 644	195	
10.	IET 13559	205	

Table 3 : IC_{50} (in mM NaCl) of root and shoot growth of ten rice cultivars in the increasing order of salt tolerance (Data taken at day 5 after incubation in dark at 27° ± 2°C).

Sl. No.	Cultivar	IC_{50} of Root	Mean IC_{50} of root	IC_{50} of Shoot	Mean IC_{50} of shoot
1.	IET 12863	90		50	
2.	CRM 30	130		55	
3.	ORM 20-22-26	135		60	
4.	IET 13427	140		70	
5.	ORM 20-17-26	145	156	75	82
6.	ORM 26-25-35	150		80	
7.	CSR 10	155		95	
8.	CR 644	200		100	
9.	IET 13410	205		105	
10.	IET 13559	210		125	

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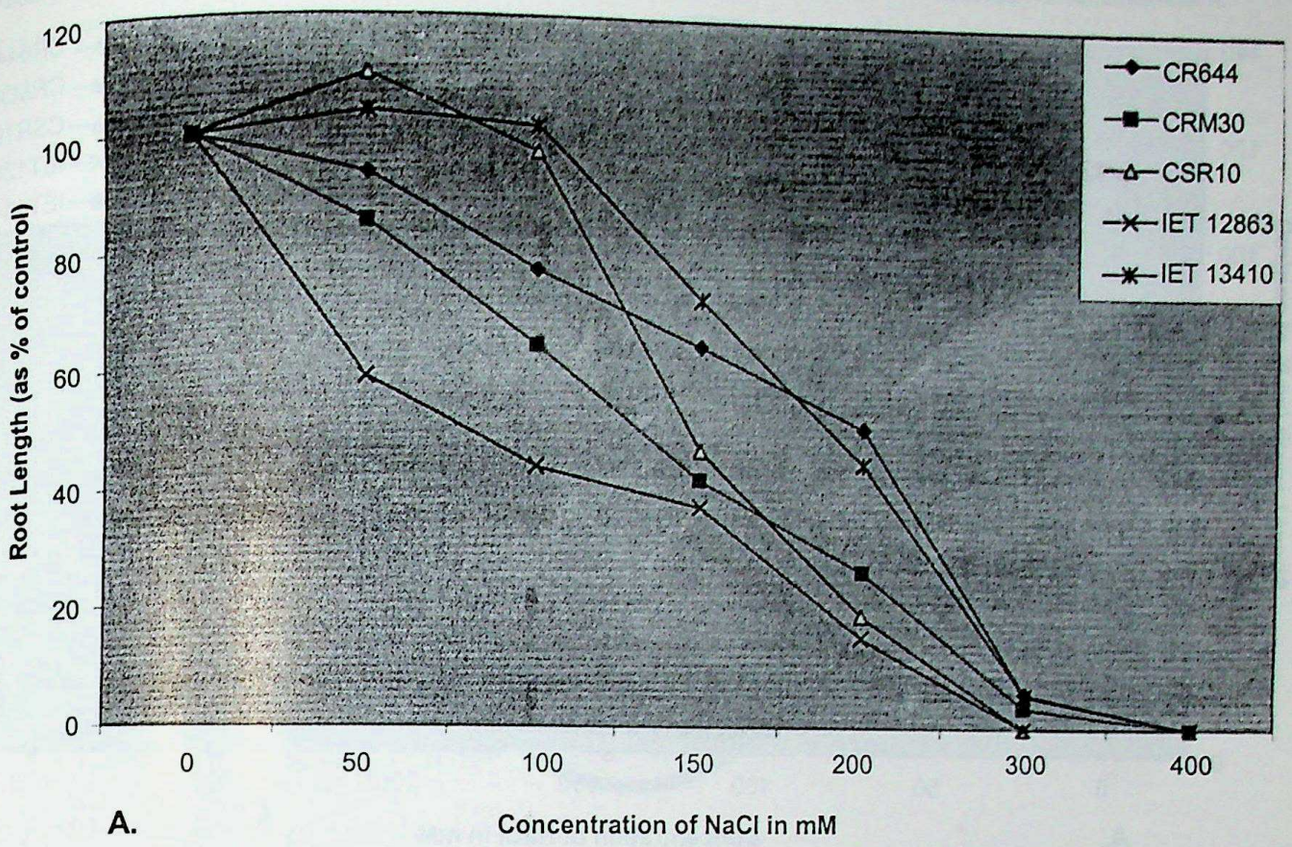
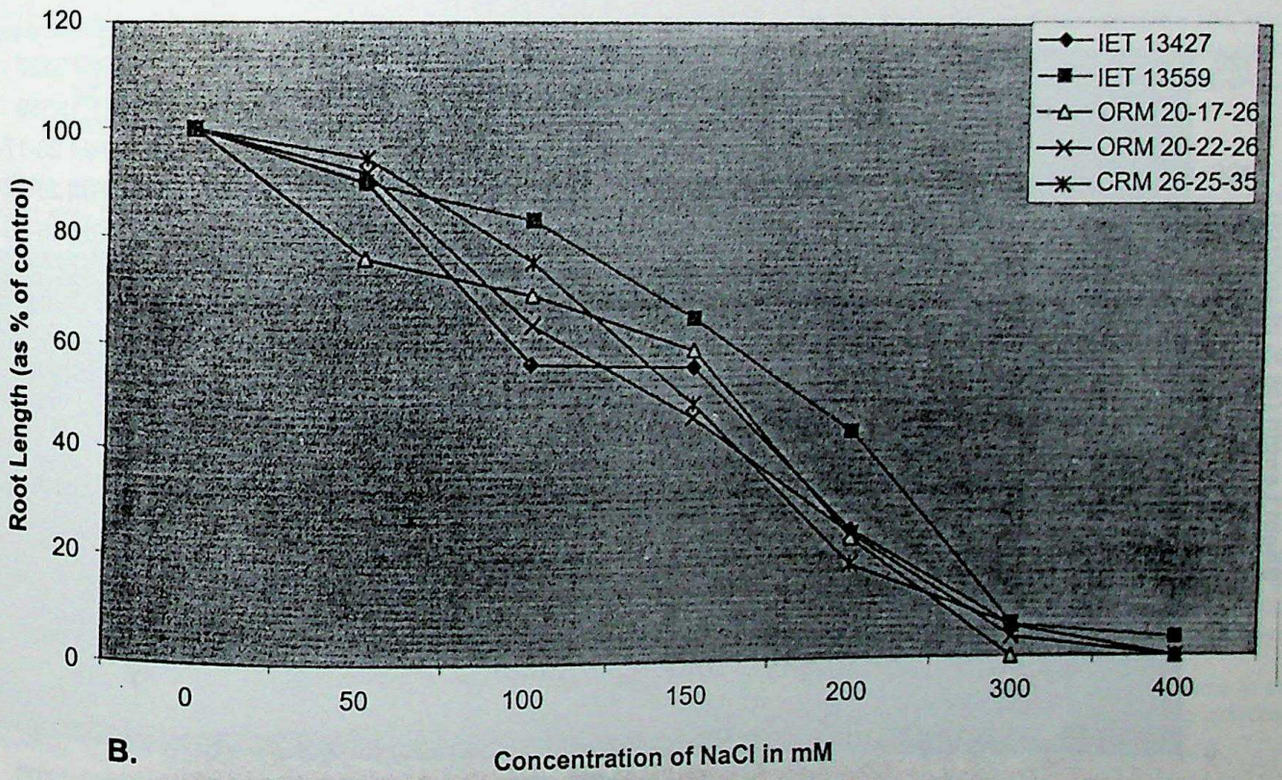


A.



B.

Fig. 1A & B. Relative root length (as % of control) of 10 rice cultivars under increasing concentrations of NaCl. Data after 5 day.

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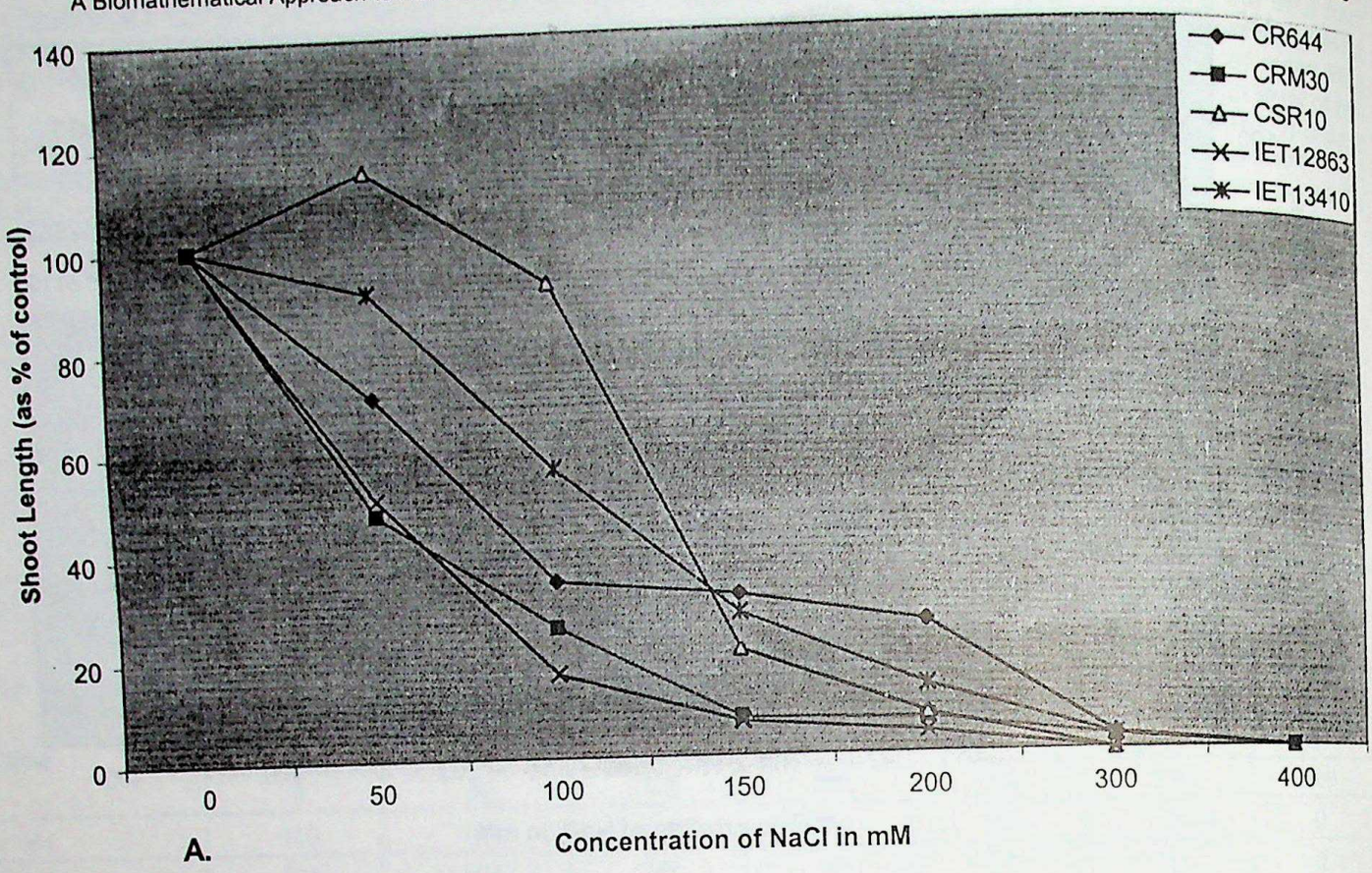

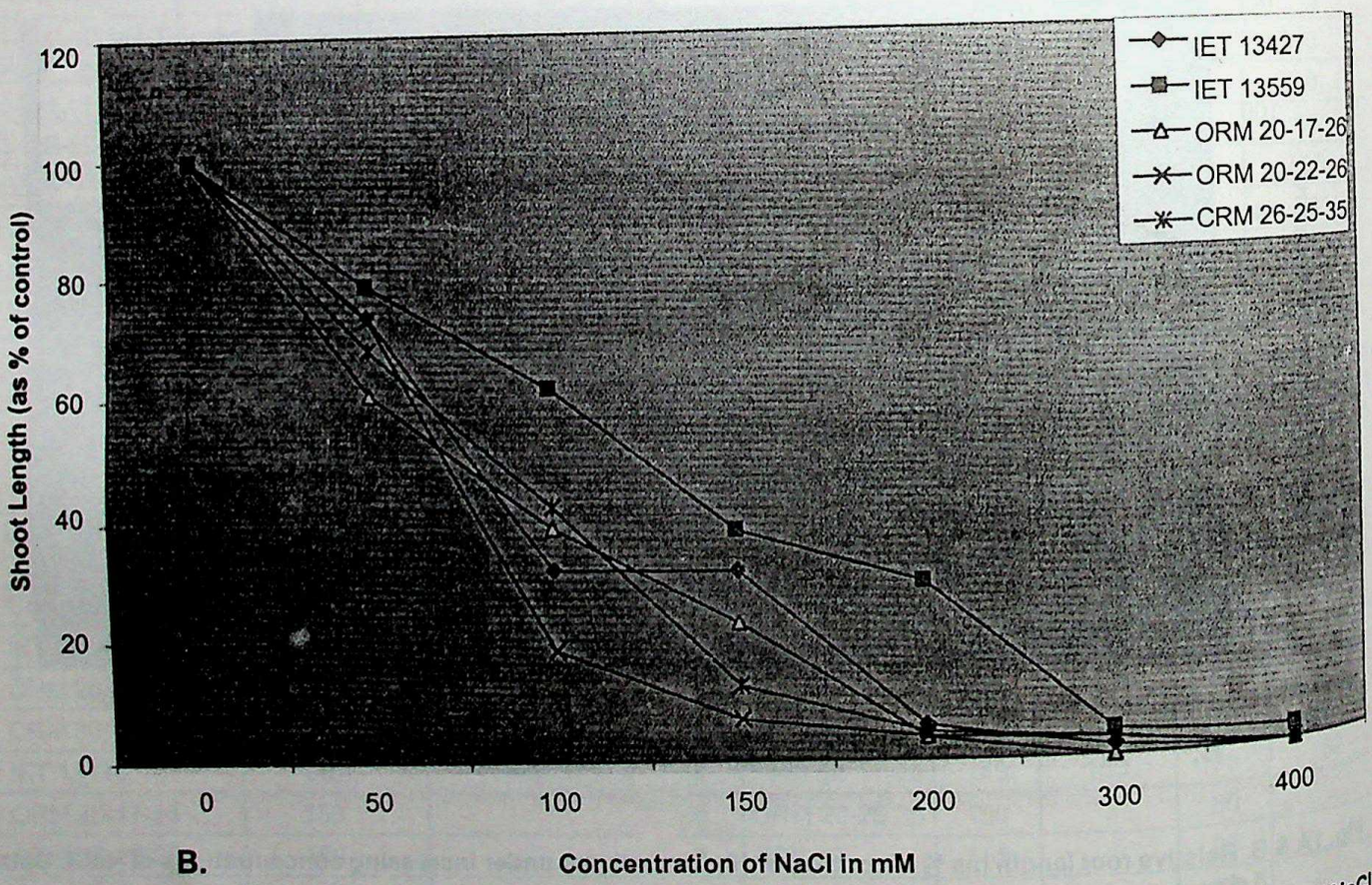

Fig. 2 A & B. Relative shoot length (as % of control) of 10 rice cultivars under increasing concentrations of NaCl. Data after 5 day.

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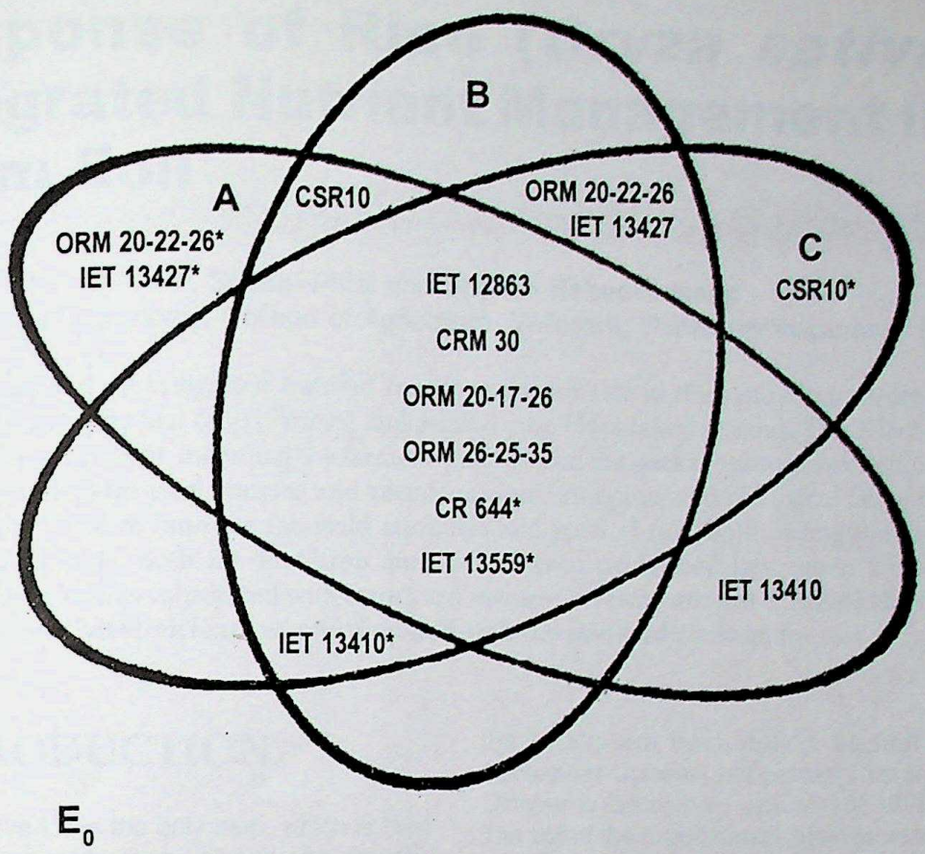

Figure 3 : Venn diagram showing level of salt tolerance of ten rice cultivars towards seed germination (A), young root growth (B) and young shoot growth (C). Cultivars with (*) indicate high level and without (*) indicate low level of salt tolerance. IC_{50} more than 156 mM for seed germination and young root growth and more than 82 mM for young shoot growth are taken as high level of salt tolerance.

seedling growth among the ten cultivars investigated. This cultivar may be grown in the saline prone areas of coastal belt of Orissa and also of India which can well combat the adverse effect of salinity on germination and seedling growth. Further, this cultivar may be taken as an effective donor parent for the construction of an effective salt resistant genotype through pyramiding the salt resistant genes following the suggestions of Yeo and Flowers (1986).

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Response of Rice (*Oryza sativa* L.) to Integrated Nutrient Management in Sandy Loam Soil

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Studies were conducted on integrated nutrient management for rice in the sandy loam tract of Kerala, the *Onattukara*, both during the first crop (*Virippu*) and second crop (*Mundakan*) seasons. The effect of six different levels of nutrient management, including the farmers' practice and the package recommendation were studied. Integration of organic (farm yard manure and vermicompost), inorganic and biological (*Azospirillum*) sources of nitrogen was observed to improve the yield attributes and yield of rice, both during the first crop season and second crop seasons. Both the first crop and second crop performed best when 25 per cent of the recommended nitrogen was substituted with farm yard manure + vermicompost + *Azospirillum*, with 54.5 per cent and 37.6 per cent yield increase when compared with the standard package for rice.

INTRODUCTION

Rice (*Oryza sativa* L.) is the only crop, which is best adapted to grow under a wide range of soil and climatic conditions. Development of suitable integrated nutrient management practices for rice grown in different agro-climatic regions plays a vital role in securing maximum monetary returns without impairing the soil health. Integrated nutrient management is a flexible approach that minimises the use of chemicals and maximises the use efficiency and farmers' profit (Sankaram, 1996). *Onattukara* is a typical agro-ecological situation in the state of Kerala. This tract has been designated as a problem area with its sandy loam soil, poor organic matter status and limited capacity to retain water and nutrients. *Onattukara* literally means the place that feeds the people during the *Onam*, the harvest festival of Kerala. Rice is the predominant crop of this region. However, the indiscriminate and continuous use of chemical fertilisers has resulted in poor soil health and the productivity of this tract has been reduced drastically. The permanent manurial trial conducted in this tract showed that chemical farming without organics steadily declined the yields. Sushamakumari *et al.* (1989) reported that the yield of rice raised with chemical fertilizers alone, declined from 2053 Kg ha^{-1} (1978-'79) to 663 Kg ha^{-1} (1984-'85). Thus any nutrient management practice, which adds organic matter to the soil, assumes paramount importance in this tract. The present study was undertaken to design a suitable integrated nutrient management practice for rice, which will help to restore and sustain soil fertility and crop productivity of the tract.

MATERIAL AND METHODS

Field experiments were conducted in the wetlands of the Rice Research Station, Kayamkulam, Kerala, located in the *Onattukara* tract, during the first crop (April-May to September-October) and second crop seasons (September - October to December - January) of 1998-99 and 1999-2000. The soil of the experimental site was sandy loam, moderately acidic in reaction (pH -5.2), low in organic carbon (0.41 %), available nitrogen (170Kg/ha) and available potassium (62Kg/ha) and medium in available phosphorus (17Kg/ha). A warm humid tropical climate tropical climate prevails over the tract, with a mean annual rainfall of 2570mm.

The experiment was laid out in a Randomised Block Design involving six nutrient management practices, replicated four times. The treatments were Farmers' practice (N_1), Package of practices recommendations of the Kerala Agricultural University (N_2), three-fourth recommended dose of nitrogen as chemical + one-fourth as FYM (N_3), three-fourth recommended dose of nitrogen as chemical + one-fourth as FYM + *Azospirillum* (N_4), three-fourth recommended dose of nitrogen as chemical + one-eighth as FYM + one-eighth as vermicompost (N_5) and three-fourth recommended dose of nitrogen as chemical + one-eighth as FYM + one-eighth as vermicompost + *Azospirillum* (N_6). The first crop of rice was direct sown; semi-dry and the second crop was a transplanted one. The paddy seeds for the first crop and second crop rice were inoculated with the biofertiliser, *Azospirillum* as per the methods suggested by Gopalaswamy *et al.* (1989a) and Gopalaswamy *et al.* (1989b) respectively. The farmers' practice of nutrient management comprised applying FYM @3t/ha uniformly during both the seasons and NPK @ 36:12:21 Kg/ha and 30:10:32 Kg/ha for the first crop and second crop rice respectively. The KAU Package of practices for rice involved the application NPK @ 70:35:35 Kg/ha and 90:45:45 Kg/ha for short duration and medium to long duration varieties respectively. Farm yard manure, when analysed, was found to contain 0.35%N, 0.60%P_2O_5 and 0.24%K_2O and

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vermicompost used for the study contained 1.60%N, 0.76%P_2O_5 and 2.71%K_2O. While the first crop season recorded a mean maximum temperature of 31.28°C, minimum temperature of 23.76°C, mean RH of 80.06 per cent and a total mean rainfall of 1472mm, the second crop season faired with a mean maximum temperature of 32.28°C, minimum temperature of 22.06°C, mean RH of 75.30 per cent and a total mean rainfall of 1027mm, over the two years.

The rice varieties chosen for the study were the short duration variety, Red Triveni (100-105 days) for the first crop season and the long duration variety, Dhanya (160-165 days) for the second crop season. The first crop was sown during the third fortnight of April and harvested during the last fortnight of July. The second crop extended from the second fortnight of August to the third fortnight of January.The cropping period was the same during both the years of study. Observations on the number of productive tillers per square meter, number of spikelets per panicle, percentage of filled grains, thousand-grain weight, grain yield, straw yield and harvest index were recorded at harvest. The nitrogen harvest index was worked out by the method suggested by Spiretz (1977). The data generated from the field trial were subjected to analysis of variance (ANOVA) as applied to Randomized Block Design (Panse and Sukhatme, 1985). Pooled analysis was done for the grain yield over the two years of study.

RESULTS AND DISCUSSION

Productive tillers per square meter : The first crop rice and second crop rice responded significantly to the different levels of nutrient management in terms of the number of panicle bearing tillers (Table 1). The farmers' practice was observed to be inferior to all the other treatments. The treatments, N_3 and N_4 were at par with the KAU Package of practices recommendations. The treatments, N_5 and N_6 which combined the favourable effects of farm yard manure, vermicompost and/ or *Azospirillum* performed better than the other treatments, during both the years of the study. Shuxin *et al.* (1991) have reported a 10 per cent increase in effective tillering due to vermicompost application. Vermicompost, in addition to its positive influence as an organic fertiliser, might have supplied more of potassium and thereby contributed to effective tillering. However, in the treatment N_6, the biological nitrogen fixer, *Azospirillum* might have complimented the effect of vermicompost. *Azospirillum*, by way of associative symbiosis might have supplied nitrogen to the plants. The favourable influence of nitrogen on the production of effective tillers has been well documented (Sheela and Alexander,1995).

Spikelets per panicle : The treatment which integrated the use of farm yard manure, vermicompost and *Azospirillum* (N_6) recorded the highest number of spikelets per panicle (Table 1). This may be attributed to the better supply of N and K through *Azospirillum* and vermicompost respectively. This finding is in conformity with the results reported by Khanda and Dixit (1996).

Percentage of filled grains : The percentage of filled grains was observed to increase progressively upto N_4 and decrease slightly thereafter, in both the first crop and second crop rice. Increase in filled grain percentage following the addition of organic matter and *Azospirillum* has been reported by Prakash *et al.* (1990). However, at higher levels of nitrogen, the percentage of filled grains was found to decrease.

Thousand grain weight : While the treatments failed to exert significant influence on the thousand grain weight of rice raised during the first crop season in the first year, significant difference could be observed the second year. The thousand grain weight of the second crop rice varied significantly among the treatments in both the years of experimentation. The thousand grain weight of first crop rice, recorded with all the treatments (N_2 to N_6), except farmers' practice (N_1), were at par during the second year. While N_6 proved to be significantly superior in terms of the thousand grain weight of second crop rice in the first year, it was at par with the other treatments during the second year. The grains yielded by the farmers' practice recorded a significantly lower grain weight compared to the other treatments. This can be attributed to the sub-optimal doses of nutrients applied in the farmers' practice. Integrated nutrient management, on the other hand has been observed to satisfy the nutrient demand of the crop, especially at the flowering and maturity stages, paving way for greater translocation to the grains, thus increasing the grain weight. This has been proven conclusively by Kiuchi and Ishizaka (1960).

Yield : The grain yield of rice showed significant response to nutrient management, with N_1 (farmers' practice) recording the lowest yield. The highest yield was recorded with N_6, the treatment that harmoniously blended the beneficial effects of farm yard manure, vermicompost and *Azospirillum*. Further, the higher grain yield at N_6 can be correlated with the increase in the number of productive tillers and spikelets per panicle at N_6. A drastic reduction in grain yield was observed in the farmers' practice. The sub-optimal dose of nutrients applied clubbed with the greater loss of nutrients, especially nitrogen from the sandy loam soil might have contributed to the poor yield. On the contrary, in the treatment N_6, *Azospirillum* and vermicompost might have ensured a better supply of nitrogen and potassium respectively, in addition to the nutrients supplied by the chemical fertilisers. The availability of phosphorus might have also been rendered better due to the solubulising effect of carbonic acids formed during the decomposition of organic matter as reported by Beresteskii *et al.* (1983).

The pooled analysis (Table 3) of grain yield showed that the treatment N_6 recorded the maximum grain yield for first and second crop rice, and was observed to be significantly superior to all the other treatments.

Harvest index (HI) : The harvest index was observed to be the highest at N_6, where in chemical fertilisers where applied in conjunction with farm yard manure, vermicompost and *Azospirillum*. This has a direct bearing on the higher grain yield recorded in the same treatment.

Nitrogen harvest index (NHI) : The NHI (Table 2) was observed to be lower with farmers' practice (N_1). The mean

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Table 1 : Yield and yield attributes of first crop and second crop rice as influenced by nutrient management.

Nutrient level	Year	Productive tillers per square meter		Spikelets per panicle		Filled grain percentage		Thousand grain weight (g)		Grain yield (Kg ha^{-1})		Harvest index	
		First crop	Second crop	First crop	Second crop	First crop	Second crop	First crop	Second crop	First crop	Second crop	First crop	Second crop
N_1	I year	180	177	47	61	76.82	76.21	25.2	25.2	1674	1665	0.283	0.246
	II year	177	205	49	63	76.39	76.32	25.2	25.1	1678	1944	0.291	0.238
N_2	I year	244	266	62	64	79.94	81.82	25.9	26.1	2439	2563	0.430	0.264
	II year	235	293	65	68	79.52	81.80	26.1	26.2	2198	2868	0.421	0.259
N_3	I year	249	285	78	77	80.91	82.65	26.0	26.3	2474	2679	0.438	0.270
	II year	267	312	77	81	80.78	82.69	26.3	26.2	2506	2955	0.439	0.264
N_4	I year	288	335	94	83	82.57	82.84	25.9	26.3	2872	3178	0.428	0.288
	II year	302	367	90	86	82.50	82.95	26.2	26.2	2834	3456	0.434	0.278
N_5	I year	323	380	99	97	80.93	81.97	25.6	26.4	3144	3575	0.455	0.302
	II year	335	386	102	95	80.32	82.10	26.0	26.3	3154	3647	0.453	0.292
N_6	I year	369	379	127	120	79.59	81.35	25.9	27.0	3608	3660	0.475	0.327
	II year	379	394	116	118	79.26	81.54	26.1	26.3	3564	3812	0.479	0.313
SEm	I year	4.032	5.964	4.155	3.527	0.126	0.177	0.186	0.128	77.968	51.316	0.0048	0.0014
	II year	4.728	5.368	1.846	2.394	0.198	0.060	0.087	0.053	40.120	38.098	0.0020	0.0020
CD	I year	12.152	16.864	12.522	10.630	0.380	0.354	-	0.384	129.843	154.651	0.014	0.004
	II year	14.247	16.178	5.564	7.216	0.598	0.182	0.263	0.160	120.909	114.816	0.006	0.005

Table 2 : Nitrogen harvest index, nutrient uptake, net returns and benefit cost ratio of first crop and second crop rice as influenced by nutrient management.

Nutrient level	Year	NHI (%)		N uptake (Kg ha^{-1})		P uptake (Kg ha^{-1})		K uptake (Kg ha^{-1})		Net returns (Rs. ha^{-1})		Benefit cost ratio	
		First crop	Second crop	First crop	Second crop	First crop	Second crop	First crop	Second crop	First crop	Second crop	First crop	Second crop
N_1	I year	36.4	32.5	52.88	62.73	11.54	12.70	81.01	96.03	2268	2126	1.15	1.04
	II year	37.1	32.0	52.59	74.80	11.74	15.61	79.33	117.96	2112	3783	1.14	1.21
N_2	I year	52.3	35.4	53.62	88.11	12.01	17.82	68.91	130.33	4090	2274	1.25	1.09
	II year	51.7	34.3	48.96	102.85	11.12	20.76	62.72	157.91	1802	6585	1.12	1.26
N_3	I year	53.1	35.4	53.60	92.59	12.00	18.72	67.66	137.46	3181	1879	1.18	1.07
	II year	52.6	35.2	54.65	103.49	12.41	21.35	69.12	157.49	3381	4936	1.20	1.18
N_4	I year	52.1	37.2	64.19	104.55	14.26	21.13	81.19	152.25	4150	2855	1.21	1.10
	II year	53.7	36.6	60.65	116.35	13.87	24.29	75.75	174.12	3268	6637	1.17	1.22
N_5	I year	54.7	39.2	66.17	111.78	14.78	22.58	81.06	159.34	5053	4875	1.25	1.16
	II year	54.0	38.3	67.03	116.98	15.27	24.09	83.06	171.83	5180	6341	1.26	1.20
N_6	I year	56.7	41.8	73.11	107.26	16.36	21.65	87.00	148.42	4618	4106	1.19	1.13
	II year	56.3	40.7	72.50	115.22	16.57	23.69	86.76	164.98	4287	6432	1.18	1.21
SEm	I year	0.394	0.093	1.261	1.775	0.242	0.360	1.543	2.646	383.3	636.3	0.025	0.029
	II year	0.292	0.065	0.902	1.343	0.222	0.316	1.234	2.005	364.1	352.9	0.024	0.014
CD	I year	1.188	0.279	3.800	5.350	0.728	1.084	4.651	7.975	1155.5	1917.4	-	-
	II year	0.886	0.197	2.720	4.048	0.668	0.952	3.720	6.042	1097.3	1063.5	0.073	0.041

NHI for the first crop rice was 51.20 per cent and 50.40 per cent respectively during the first year and second year. The treatment, N_6, which combined the beneficial effects of FYM, vermicompost and *Azospirillum*, recorded a significantly higher mean NHI for the first crop (56.50 per cent) and second crop (41.25 per cent) rice. This was even higher than the NHI (52 per cent and 34.85 per cent) recorded by the KAU package of practices recommendations (N_2).

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Table 3 : Grain yield (pooled) of first crop and second crop rice as influenced by nutrient management.

Nutrient level	Pooled grain yield (Kg ha^{-1})	
	First crop rice	Second crop rice
N_1	1676	1805
N_2	2319	2716
N_3	2490	2817
N_4	2853	3317
N_5	3149	3611
N_6	3586	3736
SEm	29.4	32.0
CD	83.3	90.4

NHI indicates the proportion of nitrogen taken up by the crop, which is utilised in producing the grain. Higher the harvest index, higher will be the NHI. In other words, NHI indicates the efficiency of translocation. The higher NHI recorded in N_6 might be the reflection of the higher HI supported by that treatment. Verma and Bhagat (1991) have reported the beneficial effect of integrating organic, inorganic and / or biological sources of nitrogen in improving the NHI of rice.

Nutrient uptake : Integrated nutrient management was observed to increase the nutrient uptake of both the first crop rice and second crop rice. Increased uptake of nitrogen was the result of the higher dry matter production and enhanced absorption of nitrogen at levels higher than N_1 (farmers' practice). The results are in conformity to those of Reddy (1988).

The favourable influence of integrated nutrient management on the nutrient uptake has been well documented. Uptake of nutrients is a function of the nutrient concentration and the total dry matter production. Thus the higher dry matter production in the treatments N_2 to N_6 might have paved way for a higher nutrient uptake as reported by Fagi and De Datta (1991). Organic matter improves the availability of phosphorus (Gaur and Singh,1982) and potassium (Manickam,1993). This might have also contributed to the higher uptake of phosphorus and potassium.

Net returns and benefit cost ratio : The first crop rice gave the highest net returns at N_5 during both the years and recorded mean returns of Rs. 5117 ha^{-1} over the two years. In the case of second crop rice, N_5 recorded the highest net returns in the first year and was at par with N_6. However, during the second year, N_4 gave the highest net returns and remained statistically at par with N_2, N_5 and N_6. The mean net return computed over the two years was found to be highest (Rs.5608 ha^{-1}) at N_5 for the second crop of rice.

The effect of different levels of nutrient management on the benefit cost ratio was observed to be significant only during the second year of study. While N_5 (three-fourth recommended dose of nitrogen as chemical + one-eighth as FYM + one-eighth as vermicompost) recorded the highest BCR for first crop rice, N_2 (KAU Package of practices recommendations) and N_4 (three-fourth recommended dose of nitrogen as chemical + one-fourth as FYM + *Azospirillum*) were at par for the second crop of rice. In sandy loam soils, as that found in the Onattukara tract of Kerala, integrated nutrient management, which combines the beneficial effects of organic matter and biofertilisers was observed to increase the yield of rice.

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Effect of Different Biofertilizer Treatments on the Germination and Growth of Wheat

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Field experiments were conducted at Dayalbagh, district Agra and Saurikh, district Kannauj (U.P.) during 2008-09 to study the effect of biofertilizers on the germination and growth of wheat, for which the seeds were treated with different combinations. Among these T_9 and T_3 treatments showed maximum germination at Dayalbagh and Saurikh site respectively. Treatments T_9 and T_8 exhibited maximum plant height at Dayalbagh and Saurikh respectively.

INTRODUCTION

Wheat is the main cereal crop of the temperate regions of the world. In India, Uttar Pradesh has the largest area under wheat cultivation. Continuous use of chemical fertilizers results in decline yield of wheat, soil fertility and increase in environmental pollution (Jin *et al.*, 1996).

Now-a-days biofertilizers are capturing the field of chemical fertilizers due to cheap availability, low cost and no side effect on land. Biofertilizers improve soil quality and provide field increments (Balyan and Indani, 2000; Pradhan, 2001). *Azospirillum* is very common soil and root bacteria. It is found in soil around roots and root surface. It fixes atmospheric nitrogen to the soil when it is added to the soil, it multiplies in millions and can supply 20-40 Kg. of Nha^{-1} per season (Swedrezynska *et al.*, 2001). Also it produces growth promoting substances like IAA, gibberellins and promotes root proliferation. It increases the rootlet density and root branching resulting in the increased uptake of minerals and water. Plant growth promoting substance like panthothenic acid, thiamine and niacin are produced by *Azospirillum* in large quantities. These substances improve the plant growth (Kapalni *et al.*, 1983). *Azatobacter* is one of the important and well known non-symbiotic nitrogen fixing bacteria. It increases 1-15% crop-yield in the well manured soil with high organic matter (Pandey, 1985). It secretes biologically active substances like thiamine, riboflaxin, IAA and gibberellic acid (Kulhari *et al.*, 1995). PSB possess the ability to bring insoluble phosphate into soluble form by secreting organic acids (Gyneshwar *et al.*, 2002).

Keeping in view, field experiments were conducted to find out the effect of biofertilizers on the germination and growth of wheat.

MATERIAL AND METHODS

Field experiments during *Rabi* 2008-09 were conducted in RBD (Random Block Designing) with three replications at Dayalbagh (Agra) and Saurikh (Kannauj). The soil of Saurikh site had pH 7.55, ECO.13dSm^{-1}, organic carbon 0.21%, available P_2O_5 15Kgha^{-1}. The Dayalbagh site had pH 7.69, ECO.21dSm^{-1}, organic carbon 0.28%, available P_2O_5 12.50 Kgha^{-1}. The experimental plots of 1m^2 were prepared and wheat variety HD2851 obtained from IARI, New Delhi for sowing.

The treatment combinations were as follows:-

T_1 - Control
T_2 - *Azospirillum* (*Azs*)+ 60 Kg Nha^{-1}
T_3 - Phosphate solubilizing bacteria (PSB) +60 Kg Nha^{-1}
T_4 - *Azatobacter* (*Azt*) +60 Kg Nha^{-1}
T_5 - PSB + 80 Kg Nha^{-1}
T_6 - *Azs* + 80 Kg Nha^{-1}
T_7 - *Azt* + 80 Kg Nha^{-1}
T_8 - PSB + *Azs* + *Azt* + 60 Kg Nha^{-1}
T_9 - PSB + *Azs* + *Azt* + 80 Kg Nha^{-1}

RESULTS AND DISCUSSION

The study demonstrated the effectiveness of biofertilizers *i.e., Azospirillum, Azatobacter*, Phosphate solubilizing bacteria (PSB) for improving the germination and growth of wheat plants.

The results for germination of seeds at Dayalbagh site depicted in Table-1 revealed that the germination percentage was found to be highest (91%) in the treatment T_9 where 80 Kg Nha^{-1} was applied in combination with PSB, *Azospirillum* and *Azatobacter*. However, the Saurikh site (Table-2) showed the significant increased in the treatment T_3 where 60 Kg Nha^{-1} was applied in combination with PSB. The difference between the combinations for maximum germination percentage may be because of difference in soil.

The lowest seed germination at both the sites was observed in the blocks treated as control, where no fertilizer was added (Table 3 and 4). Organic fertilizers was comparable in highest effectiveness to the control was 91% and 73% at

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Table 1 : Germination percentage and plant population (m^{-2}) (Dayalbagh site).

Treatments	Block 1 (B_1)	Block 2 (B_2)	Block 3 (B_3)	Mean	Germination percentage
T_1	270	290	320	293.33	73
T_2	300	330	270	300.00	75
T_3	300	290	310	300.00	75
T_4	340	315	310	321.65	80
T_5	335	325	320	326.65	82
T_6	315	340	295	316.65	79
T_7	300	325	310	311.65	78
T_8	325	340	350	338.35	85
T_9	390	360	340	363.35	91

Table 2: Germination percentage and plant population (m^{-2}) (Saurikh site).

Treatments	Block 1 (B_1)	Block 2 (B_2)	Block 3 (B_3)	Mean	Germination percentage
T_1	280	230	145	218.33	55
T_2	210	260	230	233.35	58
T_3	250	325	300	291.65	73
T_4	200	155	380	245.00	61
T_5	300	210	195	235.00	59
T_6	245	255	360	286.65	72
T_7	295	300	240	278.35	70
T_8	300	180	255	245.00	61
T_9	375	235	200	270.00	68

Table 3 : Plant height (cm) after 40 Days (Dayalbagh site).

Treatments	B_1			B_2			B_3			Mean
T_1	16	21	24	28	20	22	18	22	22	21.44
T_2	27	20	23	25	23	18	25	18	20	22.11
T_3	29	23	19	27	24	24	22	26	24	24.22
T_4	22	20	23	25	17	23	25	24	21	22.22
T_5	27	22	23	22	26	24	24	29	30	25.22
T_6	26	22	25	22	17	29	27	24	24	24.00
T_7	32	26	22	27	18	21	25	18	19	23.11
T_8	32	27	27	22	29	25	21	19	18	24.44
T_9	33	24	27	32	26	28	34	21	17	26.89

Dayalbagh and Saurikh sites respectively. The increases in germination could be attributed to the enhanced nutrient used efficiency in the presence of organic fertilizer.

Studies have shown that the plants height with the application of treatments T_9 and T_8 significantly increased at Dayalbagh and Saurikh sites respectively over the control. This might have happened due to the release of required nutrients through biofertilizers. This premise is further supported by the face that total NPK uptakes in wheat were significantly increased in response to combined application of organic and chemical fertilizers (Benbi and Biswas, 1997; Kang *et al.*, 2005). Formulation of biofertilizers and its application with different combinations for improving germination and plant growth of wheat has been novelty of this study. Thus it can be inferred from the present investigation that application of biofertilizers *i.e., Azospirillum, Azatobacter*, phosphate solubilising bacteria in combination with NPK resulted significant improvement in wheat germination and growth.

ACKNOWLEDGEMENT

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Table 4 : Plant height (cm) after 40 Days (Saurikh site).

Treatments	B_1			B_2			B_3			Mean
T_1	17	11	8	21	14	8	13	10	8	12.22
T_2	33	26	18	28	22	8	12.5	12	6	18.39
T_3	31	22	18	26	12.5	8	24	20	11	19.17
T_4	24	18	12	33	26	10	36	14	13	20.67
T_5	38	19	10	27	13	7	29	10	9	18
T_6	29	19	10	38.5	13	20	23	10	8	18.94
T_7	42	24	10	24	10	8	38	19	10	20.56
T_8	32	22	17	42	24	20	45	12	10	24.89
T_9	32.5	26	11	40	10	9	31	18	10	20.83

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T_4	22	20	23	25	17	23	25	24	21	22.22
T_5	27	22	23	22	26	24	24	29	30	25.22
T_6	26	22	25	22	17	29	27	24	24	24.00
T_7	32	26	22	27	18	21	25	18	19	23.11
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T_2	33	26	18	28	22	8	12.5	12	6	18.39
T_3	31	22	18	26	12.5	8	24	20	11	19.17
T_4	24	18	12	33	26	10	36	14	13	20.67
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Screening of Wheat (*Triticum aestivum* L.) Genotypes under Stress and Non-stress Environments

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Moisture stress is one of the major constraints in wheat (*Triticum aestivum* L.) productivity. To generate information on the effect of moisture stress on various traits which contribute for yield would be helpful in developing tolerant wheat genotypes. An experiment was conducted on a set of 28 diverse wheat genotypes including released cultivars for limited moisture conditions. The experiment was conducted under normal sown in irrigated condition and limited moisture conditions. The result revealed that days to 50% flowering and 1000 grain weight were less affected by stress conditions. The genotypes showed better performance under limited moisture stress with less reduction in grain yield. The character D.S. I could be taken as important criteria for breeding wheat genotypes suitable for stress environments.

Keywords : Wheat, Drought Susceptibility Index, Tolerant Genotypes.

INTRODUCTION

Yield potential of any variety is the combination effect of genotypes and environment interaction. Among different crop species wheat (*Triticum aestivum* L.) is one of the most important cultivated species. Now-a-days global warming and scaricity of water are important factors for the wheat production throughout the world. Howard (1924) while analyzing the factors controlling wheat production remarked that "wheat production in India is a gamble in unstable temperatures." This statement is valid even today. The cultivation of wheat is limited by (moisture stress). The present day rice- wheat cropping system and monsoonal irregularities has compelled wheat crop to face rapidly rising temperatures coupled with moisture stress during the post anthesis stages. These unfavourable environmental conditions severely affect post anthesis stages and ultimately reduce yield conciderably. It has been established that stress due to moisture can play a significant role in reducing yield and quality of wheat. Breeding for moisture stress tolerance forms an integral component of wheat breeding programme at both national and international lavel. Breeding for moisture stress tolerance in wheat cultivars requires understanding of the physiological responces of wheat crop to these stresses, which will help in identifying traits to be used as selection criteria. The present study was carried out to understand the effect on morpho-physiological traits under moisture stress conditions and identification of tolerant genotypes suitable for these environments that the yield target can be met out under changing conditions.

MATERIAL AND METHODS

The experiment was laidout at SKUAST, FOA Research Farm Chattha Jammu. During the *Rabi* season of 2005-06 and 2006-07 with 28 genotypes in which some of the genotypes were released varieties.

The experiment was sown in randomized block design with three replications and in two environmental conditions *viz,* non-stress and moisture stress condition. In moisture stress condition, one pre sowing irrigation and one irrigation at 45 days after sowing was given. In field conditions recommended agronomical practices were followed to raise a good crop. Each genotype was sown in two rows of 1.5 m length with row to row distance of 23 cm, observations were recorded on morpho-physiological characters *viz,* days to 50 per cent flowering, plant height, biomass, grain yield, harvest index, 1000 grain weight in each entry and in each replication in both conditions. The drought susceptibility index were calculated for grain yield and other attributes in stress and non-stress environments by using the formula as suggested by Fischer and Maurer (1978), D S I = (1-YD/YP)/D, where YD = mean of the genotype in stress environment, YP = mean of the genotypes in non-stress. environment, D = 1-(mean YD of all genotypes/mean YP of all genotypes). The DSI values were used to charaterise the relative tolerance of genotypes based on minimization of yield losses compared to normal environmental conditions. The differences were tested for significance by standard techniques for analysis of variance.

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Table 1 : Drought susceptibility index of different characters in bread wheat (non-stress).

S.No.	Genotype	Days to flowering	Plant height	Biomass	1000 g	Grain Yield	HI
1.	RSP-519	104	87	1.36	49.1	.286	.761
2.	RSP-520	103	85	1.83	43.5	.180	.686
3.	RSP-523	105	93	0.77	48.4	.225	.786
4.	RSP-524	104	96	0.72	33.1	.295	.797
5.	RSP-525	104	99	0.50	38.3	.330	.897
6.	RSP-526	103	97	1.47	44.4	.275	.986
7.	RSP-527	103	94	1.82	52.2	.315	.897
8.	RSP-528	101	102	2.87	49.5	.285	.899
9.	RSP-529	102	93	0.88	49.4	.200	.699
10.	RSP-530	100	97	1.78	47.5	.200	.980
11.	RSP-553	104	98	0.99	48.7	.238	.897
12.	RSP-554	103	89	0.81	54.7	.341	.789
13.	RSP-555	102	95	0.55	41.3	.565	.890
14.	PBW-175	102	98	0.38	51.3	.397	.780
15.	PBW-396	102	103	0.44	43.1	.663	.687
16.	C-306	103	102	0.70	43.6	.623	.730
17.	C-306 M	104	106	0.87	37.9	.619	.870
18.	HD 2808	105	96	0.37	44.4	.250	.390
19.	D9-9	102	105	0.45	41.6	.178	.410
20.	PBW-510	103	98	1.54	51.3	.206	.874
21.	WH-730	104	110	1.20	44.3	.280	.914
22.	PBW-561	102	105	0.86	47.7	.210	.617
23.	KO-343	101	105	0.69	50.6	.282	.640
24.	MACS-6198	100	103	1.93	43.7	.350	.810
25.	PBW-527	103	112	2.13	44.5	.297	.730
26.	WH-773	103	92	1.40	51.2	.158	.820
27.	WH-1009	104	102	1.28	46.2	.184	.780
28.	HUW-576	103	99	1.55	43.7	.154	.810
	D-value	-0.806799	-0.9579061	0.0298824	0.488	0.194918	.995178

RESULTS AND DISCUSSION

The result obtained from the analysis of variance studies showed highly significant differences among all the characters and genotypes in both environments. Further it was observed that moisture stress responded different in different genotypes.

Droughty susceptibility index (DSI) presented in the (Table-1,2) indicated (D- value) that days to flowering, 1000grain weight significantly contributing towards grain yield. Therefore in drought conditions 1000 grain weight were less affected by temperature than yield contributing trait. Similar type of results were also reported by Sharma (1993). Results of drought susceptibility index (DSI) revealed that the values of DSI were below unity with negative values for days to flowering and plant height and this was in accordance with the findings of Reynold *et al* (1996). Earlier Stafer *et al* (1996) revealed that the number of grains per panicle is negatively related to individual grain weight, which supports the results of present study.

While going through (DSI) it was noted that the breeding new varieties for this condition and more emphasis should be given to selection for high biomass, yield and 1000 grain weight. Thus selection for biomass, yield is one of the most important way to improve productivity under stress conditions.

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Table 2 : Drought susceptibility index of different characters of bread wheat (Stress).

S.No.	Genotype	Dtf	Pl.ht	Biomass	Grain yield	HI	1000grwt
1.	RSP-519	96.33	91.00	1.31	0.51	0.27	47.5
2.	RSP-520	96.66	88.66	1.11	0.34	0.36	44.1
3.	RSP-523	96.66	76.33	0.46	0.21	0.29	46.8
4.	RSP-524	96.66	99.00	0.89	0.25	0.44	38.6
5.	RSP-525	96.33	53.33	1.68	0.11	0.32	38.4
6.	RSP-526	97.33	57.33	1.21	1.14	0.39	44.6
7.	RSP-527	101.66	48.66	1.56	0.78	0.50	51.5
8.	RSP-528	96.66	52.00	1.86	0.95	0.56	52.2
9.	RSP-529	96.00	48.33	0.89	0.79	0.47	51.2
10.	RSP-530	96.33	56.00	0.87	0.36	0.53	49.7
11.	RSP-553	95.33	49.33	1.50	0.50	0.45	48.4
12.	RSP-554	95.66	40.66	2.32	0.85	0.38	50.4
13.	RSP-555	95.00	44.6	2.51	0.62	0.44	50.3
14.	PBW-175	95.66	44.66	1.02	0.25	0.44	41.7
15.	PBW-396	96.00	46.33	1.45	0.48	0.52	51.2
16.	C-306	96.33	65.66	1.62	0.41	0.46	41.5
17.	C-306 M	96.33	37.66	1.1	0.49	0.57	42.6
18.	HD 2808	96.00	52.00	1.39	0.48	0.48	37.4
19.	D9-9	95.66	45.66	1.38	0.80	0.51	42.2
20.	PBW-510	95.33	41.33	1.14	1.01	0.54	41.2
21.	WH-730	96.00	49.66	0.51	0.97	0.32	46.7
22.	PBW-561	96.33	49.33	0.48	0.79	0.35	47.1
23.	KO-343	96.33	42.00	0.57	0.99	0.32	45.9
24.	MACS-6198	95.33	40.66	0.94	0.46	0.36	50.5
25.	PBW-527	95.66	33.00	1.04	0.64	0.40	34.9
26.	WH-773	95.33	57.00	0.79	0.85	0.37	52.8
27.	WH-1009	95.66	50.00	0.69	0.37	0.41	45.9
28.	HUW-576	96.66	47.00	0.84	0.32	0.59	50.5
	D-value	0.0966996	0.4892502	-0.0308027	-0.0308029	0.446114	0.9958178

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Nutrient Optimization for Sustainable Productivity of Hybrid Maize (*Zea mays* L.) in Alfisol

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Developing soil fertility evaluation at soil series level through ASI method proposed by Portch and Hunter (2002) provides the solution to find out the limiting nutrients for sustainable productivity of any crops. Nutrient sorption study was carried out to find the deficient nutrient of the experimental soil and to fix the optimum nutrient treatment. The sorption study revealed that the N, P, K and Zn were deficient in the soil and it required as optimum nutrients of 100, 35, 75 and 4.8 mg Kg^{-1} of N, P, K and Zn for this soil. The optimum nutrient treatment (ONT) of NPK and Zn were fixed as 200:70:150 and 9.6 Kg ha^{-1} for field experiment. In each case of N, P and K there were three more levels, *viz*, a zero level, one below and another above the ONT level. The field experiment was undertaken to evaluate the nutrient optimization for sustainable productivity of hybrid maize (COHM 5) in Alfisol. The maximum plant height (149.1, 225.0 and 249.6 cm), highest drymatter production 4319 and 10962 Kg ha^{-1} were registered with the application of 250:70:150:9.6 Kg of NPK and Zn ha^{-1} at 45 and 75 DAS, respectively. Also, it influenced the yield attributes *viz* the longest cob (19.2 cm), maximum cob girth (15.2 cm), highest number of grain rows per cob (15.3), grains row^{-1} (37.1) and hundred grains weight (27.86 g). The highest grain (7908 Kg ha^{-1}) and stover yield (14.39 t ha^{-1}) were recorded for the application of 250:64:48:4.8 Kg of NPK and Zn ha^{-1} with the best net income of Rs. 35,908 ha^{-1}.

Keywords : Optimum nutrient treatment, Grain Yield, Benefit : Cost ratio.

INTRODUCTION

Maize (*Zea mays* L.) is one of the third most important cereals, next to wheat and rice in the world as well as in India. Maize is a miracle crop called as "Queen of Cereals" and is grown in more than 130 countries. The world maize cultivation area is 146 million hectares with productivity of 685 million tonnes and an average production of 4700 Kg ha^{-1}. In India, maize is cultivated in 26.7 million hectares with a production of 34 million tonnes and the average yield is 1280 Kg ha^{-1}. In Tamil Nadu maize is cultivated an area of 0.18 million hectares with a production of 0.29 million tonnes and an average productivity of 1552 Kg ha^{-1} (Season and Crop Report, 2005). By 2020, the requirement of maize for various sectors will be around 100 million tonnes, of which poultry sector needs 31 million tonnes. The concept of balanced fertilization paves the way for optimum plant nutrient supply to the full yield protential of crop and takes care of nutrient stress of soil. A systematic approach to soil fertility evaluation as proposed by Portch and Hunter (2002) envisages the optimization of fertilizer requirement for crop based on the nutrient sorption characteristics of soil. The present study is one such attempt to evolve fertilizer optima for hybrid maize (COHM 5) in benchmark soil series of Madhukkur (Mdk) belonges to the order of Alfisol.

MATERIAL AND METHODS

The experimental site located in Unchini village in Sendurai taluka of Perambalur district. The experimetal soil belongs to Madhukkur (Mdk) series of Udic Haplustalf – Alfisol. The chemical characteristics of the experimental soil was pH 6.75, EC – 0.03 dSm^{-1}, CEC – 4.3 cmol (p^+) Kg^{-1}, orgainc carbon – 0.33 per cent, available N, P and K being 148.0, 14.0 and 242.0 Kg ha^{-1}, respectively. It contained 10.0 cmol (p^+) Kg^{-1} exchangeable Ca and 2.87 cmol (p^+) Kg^{-1}exchangeable Mg. The available Zn, Fe, Cu and Mn contents were 2.06, 46.04, 4.11 and 14.3 mg Kg^{-1}, respectively.

Nutrient sorption study was carried out to find the deficient nutrient of the experimental soil and to fix the optimum nutrient treatment. The sorption study revealed that the N, P, K and Zn were deficient in the soil and it required as optimum nutrients of 100, 35, 75 and 4.8 mg Kg^{-1} of N, P, K and Zn for this soil. The optimum nutrient treatment (ONT) formed the central treatment ($N_2P_2K_2Zn$). The optimum nutrient treatment of NPK and Zn were fixed as 200:70:150 and 9.6 Kg ha^{-1} for

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field experiment. In each case of N, P and K there were three more levles, *viz.*, a zero level, one below and another above the ONT level. There is one treatment which is ONT minus Zn, except this treatment all the other treatments received Zn as in ONT. Field experiment was conducted in the farmers' field with maize (COHM5) as a test crop with thirteen treatment combinations. A blanket fertilizer treatment was included for comparison. The treatments were replicated three times in a Randomized Block Design (RBD). The crop was sown on 26.06.07 and harvested on 13.10.07. The fertilizer sources used urea, single super phosphate, muriate of potash and zinc sulphate. Full dose of P, K and Zn and one third of N were applied to maize as basal at the time of sowing. The remaining 2/3 dose of N was top dressed in 2 equal splits at 25 and 45 days after sowing. The observations on biometric and yield attributes such as plant height (45 and 75 DAS and at harvest), cob length, cob girth, grain rows cob^{-1}, number of grains row^{-1}, test weight, grain yield, dry matter production and the benefit costs: ratio were recorded and statistically analysed. The details of the treatments for this series are given in Table- 1.

RESULTS AND DISCUSSION

The plant height of maize at different states of crop growth, the grain yield and yield attributes such as cob length, cob girth, number of grain rows cob^{-1}, number of grains row^{-1} and hundred grain weight were significantly influenced. In general, the treatment receiving higher dose of N influenced the yield attributes of hybrid maize.

Growth and drymatter production : The maximum heights of plants (149.1, 225.0 and 249.6 cm) were obtained from the treatment T_4 (250:70:150:9.6 Kg of NPK and Zn ha^{-1}) followed by treatment T_3 with the application of 200:70:150:9.6 Kg of NPK and Zn ha^{-1}, which gave the next maximum heights of plants *viz.*, 137.4, 205.5 and 234.8 cm at 45, 75 DAS and at harvest (Table. 2). This might be due to continuous supply of major nutrients at the time of its critical phases of the crop growth. Similar results were observed by Singh and Singh (2006).

The highest drymatter production 4319 and 10962 Kg ha^{-1} was registered in the treatment T_4 (250:70:150:9.6 Kg of NPK and Zn ha^{-1}) at 45 and 75 DAS (Table-1). The next highest dry matter production (3517 and 10147 Kg ha^{-1}) was recorded in the treatments T_3 (250:70:150:9.6 Kg of NPK and Zn ha^{-1}) at 45 and 75 DAS. These two treatments were on per with each other at 45 and 75 DAS. Due to higher uptake of nutrients and increase in photosynthetic activity and production of more photosynthtes, the drymatter production was increased. There findings are in agreement with earlier work by Arya and Singh (2001).

Yield attributes and yield : The longest cob (19.2 cm), maximum cob girth (15.2 cm), highest number of grain rows per cob (15.3), grains row^{-1} (37.1) and hundred grains weight (27.86g) were obtained from the treatment T_4 (250:70:150:9.6 Kg of NPK and Zn ha^{-1}) followed by the optimum nutrient treatment T_3 (250:70:150:9.6 Kg of NPK and Zn ha^{-1}) (18.4 cm), (14.6 cm), (35.7) and (26.69g), respectively (Table. 3). The blanket recommendation gave lower yield attributes. The treatments were omitted with N or P or K recorded the lowest values which was closer to the absolute control. Similar findings were reported earlier by Sahoo and Mahapatra (2007) on yield attributes of maize as influenced by higher dose of NPK application. Further, these results are in close conformity with those of Singh *et al.* (2006).

The maximum grain yield (7908 Kg ha^{-1}) and stover yield (14.21 t ha^{-1}) were recorded in T_4 (250:70:150:9.6 Kg of NPK and Zn ha^{-1}) (Table. 2 & Fig.1). The application of 200:70:150:9.6 Kg of NPK and Zn ha^{-1} resulted in the next highest grain and stover yields (7210 Kg and 12.15 t ha^{-1}, respectively). The blanket recommended recorded the lower yield (5975 Kg and 9.37 t ha^{-1}) than the treatment enhanced with N, P and K. The treatment omitted with N or P or K or Zn was preformed with poor yield and which were very closer to the absolute control. The per cent increased in grain yields (32.3 and 193.1) over blanket and control were obtained from the treatment T_4 (250:70:150:9.6 Kg of NPK and Zn ha^{-1}). This was due to the increased photosynthetic activity, increase in number and elongation of cells and desirable uptake of food nutrients at all the stages of crop growth, leading to significantly higher grain yield of maize. These findings are in close conformity with the earlier findings of Reddy *et al.* (2005) and Parasuraman, (2006).

Economics : The highest dose of N as 250:70:150:9.6 Kg of NPK and Zn ha^{-1} had given the best net income of Rs. 35,908 ha^{-1} followed by the treatment with K 200:70:150:9.6 Kg of NPK and Zn ha^{-1} with the next best net income of Rs. 30,972 ha^{-1} and the benefit cost ratio of 2.52 and 2.34. The blanket recommendation with 135:62:5:50:5.5 Kg of NPK and Zn ha^{-1} recorded the net income of Rs. 23,622 ha^{-1} with the benefit cost : ratio of 2.13 (Table 3).

Table 1 : Treatment details for field experiment.

S. No.	Treatments		Nutrients doses (Kg ha^{-1})			
			N	P	K	Zn
1.	$N_0P_2K_2Zn$	:	0	70	150	9.6
2.	$N_1P_2K_2Zn$	:	150	70	150	9.6
3.	$N_2P_2K_2Zn$ (ONT)	:	200	70	150	9.6
4.	$N_3P_2K_2Zn$	:	250	70	150	9.6
5.	$N_2P_0K_2Zn$	:	200	0	150	9.6
6.	$N_2P_1K_2Zn$	:	200	52.5	150	9.6
7.	$N_2P_3K_2Zn$	:	200	87.5	150	9.6
8.	$N_2P_2K_0Zn$	:	200	70	0	9.6
9.	$N_2P_2K_1Zn$	:	200	70	112.5	9.6
10.	$N_2P_2K_3Zn$	:	200	70	187.5	9.6
11.	$N_2P_2K_2$–Zn	:	200	70	150	0
12.	Blanket	:	135	62.5	50	5.5
13.	Control	:	0	0	0	0

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Table 2 : Effect of different treatments on plant height and dry matter production of hybrid maize.

Treat. Nos.	Treatments details	Plant height (cm)			DMP (Kg ha^{-1})	
		45 DAS	75 DAS	At harvest	45 DAS	75 DAS
T_1	$N_0P_2K_2Zn$	68.2	157.6	180.2	2437	7129
T_2	$N_1P_2K_2Zn$	128.2	170.3	202.6	3312	8915
T_3	$N_2P_2K_2Zn$ (ONT)	137.4	205.3	234.8	3517	10147
T_4	$N_3P_2K_2Zn$	149.1	225.0	249.6	4319	10962
T_5	$N_2P_0K_2Zn$	88.2	164.0	185.3	2464	7584
T_6	$N_2P_1K_2Zn$	103.7	180.0	202.1	3356	8322
T_7	$N_2P_3K_2Zn$	126.6	202.6	222.3	3448	9883
T_8	$N_2P_2K_0Zn$	98.1	162.0	183.9	2514	7745
T_9	$N_2P_2K_1Zn$	110.8	173.0	197.3	3255	8487
T_{10}	$N_2P_2K_3Zn$	117.1	193.7	218.1	3509	10069
T_{11}	$N_2P_2K_2$–Zn	99.3	159.3	193.2	2714	8097
T_{12}	Blanket	111.5	165.3	197.4	3299	8113
T_{13}	Control	61.4	140.3	166.3	2333	4989
	SEd	2.86	2.16	2.43	62.70	36.43
	CD (0.05)	5.89	4.46	5.01	129.42	75.20

Table 3 : Effect of different treatments on yield parameters and grain yield of hybrid maize.

Treat. Nos.	Treatments details	Cob length (cm)	Cob girth (cm)	Grain rows cob^{-1}	Grains row^{-1}	100 grains wt (g)	Grain yield (Kg ha^{-1})	Stover yield (t ha^{-1})	Net return (Rs.ha^{-1})	B:C ratio
T_1	$N_0P_2K_2Zn$	14.0	12.3	12.7	27.4	21.76	3163	6.11	3,018	1.14
T_2	$N_1P_2K_2Zn$	16.3	13.4	14.3	31.3	25.74	6073	10.07	22,958	2.02
T_3	$N_2P_2K_2Zn$ (ONT)	18.4	14.6	14.6	35.7	26.69	7210	12.15	30,972	2.34
T_4	$N_3P_2K_2Zn$	19.2	15.2	15.3	37.1	27.86	7908	14.21	35,908	2.52
T_5	$N_2P_0K_2Zn$	14.8	12.4	13.3	28.6	23.41	3740	7.85	7,134	1.33
T_6	$N_2P_1K_2Zn$	15.7	13.5	14.2	30.8	24.59	6268	10.04	24,268	2.07
T_7	$N_2P_3K_2Zn$	17.8	14.5	14.5	34.3	26.08	6961	11.12	28,543	2.21
T_8	$N_2P_2K_0Zn$	16.1	12.4	13.2	29.0	23.47	3926	6.80	7,509	1.34
T_9	$N_2P_2K_1Zn$	17.0	14.3	14.1	30.4	25.24	6193	9.78	23,582	2.04
T_{10}	$N_2P_2K_3Zn$	18.2	14.5	14.5	34.4	26.10	7094	11.98	29,927	2.28
T_{11}	$N_2P_2K_2$–Zn	15.3	13.1	13.1	30.1	23.18	5785	9.15	21,343	1.98
T_{12}	Blanket	16.0	13.3	13.5	30.7	25.11	5975	9.37	23,622	2.13
T_{13}	Control	13.4	11.6	12.1	24.5	20.48	2698	5.17	3,136	1.18
	SEd	0.32	0.12	0.09	0.25	0.30	114.81	0.22		
	CD (0.05)	0.65	0.24	0.19	0.53	0.61	236.96	0.45		

CONCLUSION

The grain yield increased (32.3 per cent) with the application of 250:70:150:9.6 Kg of NPK and Zn ha^{-1} followed by the application of 200:70:150:9.6 Kg of NPK and Zn ha^{-1} (20.7 per cent) over blanket recommendation. Significant economic yield was obtained in the ONT + Zn in Madhukkur soil series over and above the blanket recommendation presently followed for hybrid maize. Developing soil fertility evaluation at series level through ASI method for hybrid maize (COHM 5) was found to be beneficial to farmers. Hence, the recommendation was made for hybrid maize (COHM 5) grown in this soil series as Kg 250:70:150:9.6 Kg of NPK and Zn ha^{-1}.

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Effect of Different Supplements on Spent Mushroom Substrate (SMS) of *Pleurotus* species

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The species *Pleurotus sajor-caju* and *Pleurotus florida* were cultivated on paddy straw beds with and without supplements *viz*, wheat bran, rice bran, gram flour, neem cake and inorganic fertilizers. The paddy straw (1 Kg dry or about 3.5 Kg wet weight) and spawn @ 3% wet weight of paddy straw were common to all the treatments. The supplements were added on dry weight basis. One gram digested sample was used for estimation of phosphorus, patassium, calcium sulphur and iron content. The results of calcium and iron were confirmed on Perkin Elmer's AAnalyst 200 modal of Atomic Absorption Spectrophotometer (AAS). The data on nutritionally important element content revealed that the oven dried mushroom contained 1.58 to 2.42 per cent phosphorus, 2,30 to 3.18 per cent potassium, 22.7 to 44.0 mg calcium, 178.8 to 257.9 mg sulphur and 11.1 to 15.4 mg iron per 100 g of mushroom.

Keywords : AAS, Mineral element, *Pleurotus florida, Pleurotus sajor-caju,* Supplement, Paddy straw.

INTRODUCTION

Spent mushroom substrate (SMS) which is left after the harvest of mushroom crop has been found to be very good for improving soil fertility and productivity, preparation of organic manure, bioremediation of polluted soil water, regrowing of mushrooms, biogas production, biocontrol agent for plant diseases and as animal feed (Gupta, 2006). Further the organic matter degraded by mushroom (SMS) can be utilized for quick production of manure. Several workers have used different raw and enriched substrates and studied their effect on yield of mushroom.

The information on the effect of enriched substrate is not available. The effect of different supplements on mineral composition of spent mushroom substrate (SMS) of two varieties of oyster mushroom grown under Konkan agro-climatic condition is therefore proposed.

MATERIAL AND METHODS

The Mushroom spawn of two species of Oyster mushroom *viz.*, *Pleurotus sajor-caju* and *Pleurotus florida* were cultivated on paddy straw beds prepared with and without supplements in a room previously disinfected with 2 per cent formalin. The supplements *viz.*, wheat bran, rice bran, gram flour, neem cake and inorganic fertilizers were sterilized in autoclave at 1 Kg/cm^2 pressure for one hour. The polypropylene bags (35 cm x 55 cm) were sterilized by dipping them in 2 per cent formalin solution and filled with pasteurized wet paddy straw (approx. 3.5 Kg wet weight), spawn and supplements. This spawn and supplement were added along the periphery of the bed in five layers as per standard procedures.

The supplements used in the study and their mineral composition along with paddy straw is given in Table 1.

The paddy straw (1 Kg dry or about 3.5 Kg wet weight) and spawn @ 3% wet weight of paddy straw were common to all the treatments. The supplements were added on dry weight basis as per the treatment details given below.

The experiment was started on 24th September 2005 in Department of Agricultural Chemistry and Soil Science, College of Agriculture, Dapoli (MS) under standard recommended condtions.

The mineral elements *viz.*, nitrogen, phosphorus, potassium, calcium, sulphur and iron in paddy straw and supplement samples at the beginning of the experiment and in the mushroom bed samples after the third harvest of mushroom were determined after preparing acid extracts as per the standard procedures.

The results of the calcium and iron were also confirmed by using Perkin Elmer's AAnalyst 200 model of Atomic Absorption Spectrophotometer (AAS).

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RESULTS AND DISCUSSION

The data on the effect of different supplements on mineral composition of *Pleurotus* harvested spent mushroom substrate are presented in Table 3.

The data on Nitrogen content of *Pleurotus* harvested spent mushroom substrate (SMS) revealed that the wheat bran treatment (T_3) recorded significantly higher nitrogen content (1.05%) as compared with rest of the treatments. The SMS of *Pleurotus florida* recorded significantly higher nitrogen content (0.86%) as compared with that of *Pleurotus sajor–caju* (0.74%). The interactions of treatment and species combinations were not significant. The values for nitrogen content of SMS (paddy straw) reported by Dhanda *et al.* (1996) agreed closely with the values observed in present study. However, they observed higher nitrogen content (0.86%) in *Pleurotus sajar–caju* harvested SMS as compared with the SMS of *Pleurotus florida*, which was just opposite to our observations. Gupta *et al.* (2004) reported the nitrogen content values of spent mushroom substrate in the range from 0.07 to 2.17 per cent using wheat straw as substrate.

The fertilizer treated SMS (T_6) recorded significantly higher phosphorus content (1.10%) as compared with all other treatments. Alike nitrogen, the *Pleurotus florida* SMS recorded significantly higher phosphours content (0.53%) as compared with the SMS of *Pleurotus sajor–caju*. Among the different treatment combinations the fertilizer–*Pleurotus florida* (V_2T_6) treatment recorded significantly higher phosphorus content than all other treatment combinations expcept V_1T_6, which was at par with it. Gupta *et al.* (2004) reported that the phosphorus content values of SMS ranged from 0.27 to 1.39 per cent, which were in accordance with the phosphorus content values of SMS obtained in present investigation.

The effect due to different supplements on potassium content of *Pleurotus* harvested SMS was significant. The potassium content of SMS was found to decrease significantly under different treatments except fertilizer treatment (T_6) which was at par with control (T_1). Alike nitrogen and phosphorus, *Pleurotus florida* harvested SMS recorded significantly higher potassium content (0.97%) as compared with *Pleurotus sajor–caju* (0.55%). The interaction differences in potassium content of SMS were also significant. The potassium content values (0.22 to 0.50%) of SMS recorded by Gupta *et al.* (2004) were slightly lower than the potassium content values of *Pleurotus* harvested SMS obtained in present study.

The wheat bran treated SMS (T_3) recorded significantly higher sulphur content (0.17%) than all other treatments. The treatment T_2 was significantly higher in sulphur content as compared with control treatment (T_1). The effect due to species on SMS was not significant. Among the different treatment combinations, the wheat bran–*Pleurotus florida* (V_2T_3) recorded significantly higher sulphur content (0.18%) as compared with all treatment combinations, except wheat bran–*Pleurotus sajor–caju* treatment combination (V_1T_3) which was at par with it.

The effect of different supplements on calcium content of *Pleurotus* harvested SMS was not significant. The calcium content was however affected significantly by species being higher in SMS of *Pleurotus sajor–caju*. The interation effect on calcium content of SMS was also significant.

The neem cake treated SMS (T_5) recorded significantly higher iron content (430 mg/ 100 g) as compared with all other treatments. All other treatments were at par with control except wheat bran treatment (T_3). The comparison of species revealed that alike calcium the *Pleurotus sajor–caju* harvested SMS recorded significantly higher iron content (333 mg/100 g) as compared with SMS of *Pleurotus florida* (318 mg/100 g). The interaction of *Pleurotus sajor–caju* and neem cake (V_1T_5) produced the higher amount of iron (453 mg/100 g) in SMS and it was significantly highest than all other treatments.

Improvement in mineral composition of paddy straw : There are reports which indicate that the cultivation of mushroom improves the feed value of substrate (SMS). The mushroom bed at the beginning of the experiment and after harvest of the mushroom was therefore analysed for its mineral content and the data are presented in Table 4 (A) and (B).

The nitrogen content of *Pleurotus sajor–caju* mushroom bed was found to increase by about 1.3 times and as 1.5 times in case of *Pleurotus florida* as compared with its initial mean nitrogen content (0.57%) and was nearly comparable. Dhanda *et al.* (1996) observed that the nitogen content (0.57%) of the straw got almost doubled after *Pleurotus sajor–caju* and *Pleurotus florida* harvest (1.07 and 1.02%), respectively. The results reported by Dhanda *et al,* (1996) were in accordance with the nitrogen content values of SMS obtained in present investigation. Similarly, Jadhav (1998) reported that the concentration of nitrogen in spent substrate increased with reduction in C : N ratio. Silanikove *et al.* (1988) reported that the oyster mushroom degraded the lignin, increased protein value and digestibility of SMS consisting of paddy straw and wheat straw and supplements like cotton seed, cotton seed cake and mustard cake.

The phosphorus content of *Pleurotus sajor–caju* mushroom bed was found to increase by 2.05 times as compared with its initial mean phosphorus content (0.17%). The increase in phosphorus content of mushroom bed in case of *Pleurotus florida* was 3.11 times and was higher than *Pleurotus sajor–caju* harvested SMS. The cultivation of *Pleurotus* species thus increased the phosphorus content in SMS by 2 to 3 times.

The potassium content of SMS was thus adversely affected by cultivation of *Pleurotus* species. The adverse effect on potassium content of SMS was greater in case of *Pleurotus sajor–caju* as compared with *Pleurotus florida*.

The sulphur content of SMS was decreased by the cultivation of both the species and the decrease was negligible.

The calcium content of *Pleurotus sajor–caju* harvested mushroom bed was found to increase by about 1.6 times as

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Table 1 : Mineral, compostition of supplements and paddy straw used in the study.

Sample	N (%)	P (%)	K (%)	S (mg/100g)	Ca (mg/100g)	Fe (mg/100g)
Paddy Straw	0.49	0.11	1.59	169.30	554.00	284.0
Rice bran	1.95	1.36	1.78	180.00	90.00	19.0
Wheat bran	2.21	1.29	1.23	210.00	148.00	20.88
Gram flour	3.32	0.33	0.72	160.00	56.00	9.10
Neem cake	2.50	1.00	1.40	1070.00	90.00	15.00
Urea	46.00	—	—	—	—	—
KH_2PO_4	—	22.79	28.68	—	—	—

Table 2 : Treatment details.

Treatment	Description
T_1	Control
T_2	10% Rice bran
T_3	10% Wheat bran
T_4	5% Gram flour
T_5	2% Neem cake
T_6	Fertilizer mixture (Urea 1.15 g + KH_2PO_4 4.38 g + Cellulose powder 94.47 g)

compared to its initial mean calcium content (0.53%). The increase in calcium content of *Pleurotus sajor–caju* harvested mushroom bed was very much comparable with the *Pleurotus florida* harvested bed as calcium content in case of *Pleurotus florida* harvested mushroom bed was also increased by 1.5 times.

On an average the increase in calcium content of SMS caused by both the species was lowest in fertilizer treatment (T_6) and highest in gram flour treatment (T_4).

The iron content was found to increase by 1.21 times in *Pleurotus sajor–caju* harvested mushroom bed and 1.16 times in *Pleurotus florida* harvested mushroom bed, as compared with the initial iron content (272.9 mg/100 g). In fertilizer treatment (T_6), on the other hand, a decrease in iron content of SMS was observed due to cultivation of *Pleurotus sajor–caju*. Both the *Pleurotus* species increased the iron content of SMS and the increase (59.6 mg/100 g) was greater with *Pleurotus sajor–caju* than *Pleurotus florida* (44.7 mg/ 100 g).

The comparison of initial and final mineral composition of SMS revealed that the mushroom cultivation increased the contents of nitrogen, phosphorus, calcium and iron in the paddy straw used as a substrate with and without supplements. The increase in mineral content varied with species. The *Pleurotus sajor–caju* cultivated bed showed greater increase in the content of calcium and iron, while *Pleurotus florida* cultivated beds showed greater increase in nitrogen and phosphorus contents.

The mushroom cultivation did not seem to benefit the potassium and sulphur contents of the paddy straw. The adverse effect of mushroom cultivation on sulphur content was negligible while it was profound in case of potassium content of paddy straw after mushroom harvest. The beneficial effect of mushroom cultivation on feed value of mushroom substrate has been reported by Shetty and Moorthy (1981). They obtained about 50 per cent increase in crude protein content of SMS when inoculated with *Pleurotus sajor–caju*. Similarly, the beneficial effect of mushroom cultivation on mushroom substrate has also been reported by Dhanda *et al.* (1996).

They observed that the nitrogen content (0.575%) of straw got almost doubled after the first flush of *Pleurotus florida* and *Pleurotus sajor-caju*. They further reported that the total minerals in the spent paddy straw were also increased due to cultivation of the *Pleurotus* species. The results observed in the present study agreed well with the findings, that the spent straw after the harvest of mushroom has superior feed value as reported by Dhanda *et al.* (1996).

Similar results of increase in feed value of spent mushroom substrate has been reported by Mudgal *et al.* (1981) and Mandhare *et al.* (2003).

Gupta *et al.* (2005) found that mushroom spent paddy straw was beneficial in reducing the C : N ratio and enhanced the rate of composting. They further found that the compost prepared out of spent paddy straw was richer in NPK as compared with the compost prepared out of fresh paddy straw, as the SMS is richer in nutrients as compared with fresh paddy straw.

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Table 3 : Effect of different supplements on mineral content of spent mushroom substrate (SMS).

Treatment	Nitrogen (%)			Phosphorus (%)			Potassium (%)			Sulphur (%)			Calcium (g/100 g)			Iron (mg/100 g)		
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1–Control	0.41	0.50	0.46	0.15	0.44	0.30	0.56	1.22	0.89	0.12	0.13	0.13	0.96	0.77	0.86	301	292	297
T_2–Rice bran	0.77	0.86	0.81	0.35	0.52	0.44	0.43	0.99	0.71	0.14	0.16	0.15	0.77	0.91	0.84	325	273	299
T_3–Wheat bran	0.97	1.12	1.05	0.19	0.60	0.40	0.47	0.84	0.66	0.17	0.18	0.17	0.96	0.80	0.88	344	336	340
T_4–Gram flour	0.82	0.99	0.91	0.14	0.30	0.22	0.50	0.94	0.72	0.14	0.14	0.14	0.97	0.85	0.91	301	302	302
T_5–Neem cake	0.71	0.84	0.78	0.17	0.17	0.17	0.47	0.78	0.63	0.13	0.11	0.12	0.81	0.77	0.79	453	407	430
T_6–Fertilizer	0.76	0.82	0.79	1.09	1.12	1.10	0.87	1.06	0.97	0.12	0.14	0.13	0.78	0.73	0.75	271	295	283
Mean	**0.74**	**0.86**	**0.80**	**0.35**	**0.53**	**0.44**	**0.55**	**0.97**	**0.76**	**0.14**	**0.14**	**0.14**	**0.88**	**0.80**	**0.84**	**332**	**318**	**325**
S.E.± (T)	0.020			0.013			0.041			0.002			0.03			5.060		
C.D. @ 1%	**0.081**			**0.052**			**0.164**			**0.010**			**N.S.**			**20.163**		
S.E. ± (V)	0.012			0.008			0.024			0.001			0.02			2.921		
C.D. @ 1%	**0.047**			**0.030**			**0.094**			**N.S.**			**0.07**			**11.641**		
S.E. ± (VxT)	0.029			0.018			0.058			0.003			0.04			7.156		
C.D. @ 1%	**N.S.**			**0.073**			**0.231**			**0.014**			**0.17**			**28.515**		

Table 4 (A). Improvement in mineral composition of paddy straw by *Pleurotus* species cultivation.

Treatment	Nitrogen (%)						Phosphorus (%)						Potassium (%)					
	V_1			V_2			V_1			V_2			V_1			V_2		
	Initial	Final	Increase/ decrease	Initial	Final	Increase/ decrease	Initial	Final	Increase/ decrease	Initial	Final	Increase/ decrease	Initial	Final	Increase/ decrease	Initial	Final	Increase/ decrease
T_1–Control	0.49	0.41	-0.08	0.49	0.50	+0.01	0.11	+0.15	0.04	0.11	+0.44	+0.33	1.59	0.56	-1.03	1.59	1.22	-0.37
T_2–Rice bran	0.62	0.77	+0.15	0.62	0.86	+0.24	0.22	0.35	+0.13	0.22	0.52	+0.30	1.61	0.43	-1.18	1.61	0.99	-0.62
T_3–Wheat bran	0.65	0.97	+0.32	0.65	1.12	+0.47	0.22	0.19	-0.03	0.22	0.60	+0.38	1.56	0.47	-1.09	1.56	0.84	-0.72
T_4–Gram flour	0.62	0.82	+0.20	0.62	0.99	+0.37	0.12	0.14	+0.02	0.12	0.30	+0.18	1.55	0.50	-1.05	1.55	0.94	-0.61
T_5–Neem cake	0.53	0.71	+0.18	0.53	0.84	+0.31	0.13	0.17	+0.04	0.13	0.17	+0.04	1.59	0.47	-1.12	1.59	0.78	-0.81
T_6–Fertilizer	0.49	0.76	+0.27	0.49	0.82	+0.33	0.19	1.09	+0.90	0.19	1.12	+0.93	1.56	0.87	-0.69	1.56	1.06	-0.50
Mean	**0.57**	**0.74**	**+0.17**	**0.57**	**0.86**	**+0.29**	**0.17**	**0.35**	**+0.18**	**0.17**	**0.53**	**+0.36**	**1.58**	**0.55**	**-1.03**	**1.58**	**0.97**	**-0.61**

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Table 4 (B). Improvement in mineral composition of paddy straw by Pleurotus species cultivation.

Treatment	Sulphur (%)						Calcium (%)						Iron (mg/100 g)					
	V_1			V_2			V_1			V_2			V_1			V_2		
	Initial	Final	Increase/ decrease	Initial	Final	Increase/ decrease	Initial	Final	Increase/ decrease	Initial	Final	Increase/ decrease	Initial	Final	Increase/ decrease	Initial	Final	Increase/ decrease
T_1–Control	0.16	0.12	-0.04	0.16	0.13	-0.03	0.55	0.96	+0.41	0.55	0.77	+0.22	284.0	301.4	+17.4	284.0	291.5	+7.5
T_2–Rice bran	0.17	0.14	-0.03	0.17	0.16	-0.01	0.51	0.77	+0.26	0.51	0.91	+0.40	259.9	324.6	+64.7	259.9	273.1	+13.2
T_3–Wheat bran	0.17	0.17	0.00	0.17	0.2	+0.01	0.52	0.96	+0.44	0.52	0.80	+0.28	259.9	349.9	+84.0	259.9	336.4	+76.5
T_4–Gram flour	0.17	0.14	-0.03	0.17	0.14	-0.03	0.1	0.97	+0.44	0.53	0.85	+0.32	270.9	300.8	+29.9	270.9	302.2	+31.3
T_5–Neem cake	0.19	0.13	-0.06	0.19	0.11	-0.08	0.54	0.81	+0.27	0.54	0.77	+0.23	278.7	453.3	+174.6	278.7	407.2	+128.4
T_6–Fertilizer	0.15	0.12	-0.1	0.15	0.14	-0.01	0.55	0.78	+0.23	0.55	0.72	+0.17	284.0	270.7	-13.3	284.0	296.0	+11.0
Mean	**0.17**	**0.14**	**-0.03**	**0.17**	**0.14**	**-0.03**	**0.53**	**0.88**	**+0.33**	**0.53**	**0.80**	**+0.27**	**272.9**	**332.5**	**+59.6**	**272.9**	**317.6**	**+44.7**

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Effect of Cutting Schedule and Nutrient Management on Contents and Uptakes of Major Nutrients by Forage Oats (*Avena sativa* L.)

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A field experiment entitled "Response of forage oats (*Avena sativa* L.) to cutting schedule and nutrient management under Saurashtra region" was conducted at Instructional Farm, Junagadh Agricultural University, Junagadh on clayey soil during *rabi* seasons of 2006-07 and 2007-08. The results of the experiment indicated that the treatment of cutting schedule one cut (C_1) produced significantly higher nitrogen and phosphorus content as compared to C_2 (two cuts), while cutting schedule C_2 (two cuts) produced significantly higher nitrogen, phosphorus and potassium uptakes than those of one cut (C_1). Treatment D_4 (125% RDF) significantly recorded higher nitrogen; phosphorus and potassium contents and uptakes than rest of fertilizer levels. The application of 15 t FYM ha^{-1} (F_1) significantly recorded the highest nitrogen, phosphorus and potassium contents and uptakes as compared to F_0 (without FYM) during both the years and in pooled data, while potassium content was not found significant in pooled results.

INTRODUCTION

Oats (*Avena sativa* L.) is versatile *rabi* cereal fodder crop of India. It is rich in energy, protein, vitamin B, phosphorus and iron and it is used as a green fodder, hay and silage. Its popularity as a fodder crop is increasing because of excellent growth habit, quick regrowth and high nutritive value. Nitrogen plays an important role in synthesis of chlorophyll and amino acid, which contributes to the building units of protein and the growth of plants. Insufficient nitrogen may reduce yield drastically and deteriorates the quality of crop produce. Phosphorus plays an important role in protein metabolism, seed formation, early flowering and quality of economic produce. Application of heavy doses of inorganic fertilizer is not a sound management practice because of high cost of fertilizers and nitrate toxicity due to high nitrogen fertilizer application. Use of FYM minimizes the use of chemical fertilizer and improves soil physical properties. Therefore, the research work on "Response of forage oats (*Avena sativa* L.) to cutting schedule and nutrient management under Saurashtra region" was carried out during *rabi* seasons of 2006-07 and 2007-08 at Instructional Farm, College of Agriculture, JAU, Junagadh.

MATERIAL AND METHODS

The climate of this region is sub-tropical. In general, monsoon is warm and moderately humid, commences by third weak of June and retreats by the end of September with an average annual rainfall of about 935.4 mm in 39 rainy days during last 10 years of 1995 to 2005. Winters is fairly cold and dry (temperature ranging from 9.1 to 21.0 °C minimum and 35.2-23.6 °C maximum during period of experiment), while the summer is quite hot and dry. The soil of experimental plot was clayey in texture, calcareous in nature and slightly alkaline in reaction. The soil was medium in available N and P and rich in available K.

The experiment comprising of all possible treatment combinations of two levels of cutting schedule (C_1- One cut at 75 DAS and C_2- Two cuts - one at 55 DAS and second at 90 DAS); Four levels of inorganic fertilizer (D_1- 50 % RDF, D_2-75 % RDF, D_3-100 % RDF and D_4-125 % RDF) and two levels of organic manure (with and without application of FYM) were tried in factorial randomized block design with four replications. As per treatment, well decomposed FYM @ 15 t/ha was applied after bund formation of the plot. Major nutrient contents of FYM were 0.53 and 0.51 % N, 0.22 and 0.25 % P and 0.74 and 0.72 % K during year 2006-07 and 2007-08, respectively. In case of Recommended Dose of Fertilizer (80:30:00 N: P_2O_5: K_2O Kg ha^{-1}), Urea and Diammonium Phosphate were used as source of nitrogen and phosphorus. Half of nitrogen and full dose of phosphorus were applied as basal as per treatments. The remaining half dose of nitrogen was top dressed at 30 days after sowing for one cutting schedule, while in case of two cutting schedule 25% nitrogen was top dressed at 30 days after sowing and remaining 25% nitrogen after first cut.

The observations to be recorded as plant height (cm), number of tillers per plant, number of leaves per plant in 5 plants per plot, green forage and dry fodder yield (Kg) from net plot, crude protein (%), Crude protein yield, NPK content

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Table 1 : Nitrogen, phosphorus and potassium content (%) in dry fodder of oats as influenced by cutting schedule and nutrient management.

Treatments / Year	Nitrogen			Phosphorus			Potassium		
	2006-07	2007-08	Pooled	2006-07	2007-08	Pooled	2006-07	2007-08	Pooled
Cutting									
C_1 - One cut	1.84	1.76	1.80	0.223	0.229	0.226	0.98	1.15	1.06
C_2 - Two cuts	1.64	1.51	1.58	0.209	0.218	0.214	0.95	1.16	1.06
S.Em. +	0.03	0.02	0.02	0.002	0.003	0.002	0.01	0.01	0.01
C.D. at 5%	0.09	0.06	0.06	0.006	0.008	0.005	NS	NS	NS
Levels of fertilizer (RDF-80: 30: 00 N: P_2O_5: K_2O Kg ha^{-1})									
D_1 - 50 % RDF	1.59	1.45	1.52	0.191	0.200	0.195	0.88	1.05	0.97
D_2 - 75 % RDF	1.71	1.66	1.68	0.210	0.217	0.213	0.95	1.14	1.04
D_3 - 100 % RDF	1.81	1.71	1.76	0.227	0.234	0.231	1.00	1.19	1.09
D_4 - 125 % RDF	1.86	1.73	1.80	0.238	0.243	0.240	1.03	1.25	1.14
S.Em. +	0.04	0.03	0.03	0.003	0.004	0.002	0.02	0.02	0.01
C.D. at 5%	0.13	0.09	0.08	0.008	0.011	0.007	0.05	0.05	0.04
Levels of FYM									
F_0- Without FYM	1.67	1.58	1.62	0.211	0.218	0.215	0.94	1.11	1.02
F_1- 15 t ha^{-1} FYM	1.82	1.70	1.76	0.221	0.229	0.225	0.98	1.21	1.10
S.Em. +	0.03	0.02	0.02	0.002	0.003	0.002	0.01	0.01	0.02
C.D. at 5%	0.09	0.07	0.06	0.006	0.008	0.005	0.04	0.04	NS
Interactions (C.D. at 5%)									
C x D	NS	NS	NS	0.011	0.016	0.010	NS	NS	NS
C x F	NS	NS	NS	NS	NS	NS	NS	NS	NS
D x F	NS	NS	NS	NS	NS	NS	NS	NS	NS
C x D x F	NS	NS	NS	NS	NS	NS	NS	NS	NS
C.V. %	10.70	7.66	9.40	5.160	7.050	6.210	7.31	6.47	6.86

at the time of cutting and economics (net realization Rs./ha and B:C ratio) were worked out after the harvest of the crop.

As plant analysis representative samples of dry fodder were collected from each net plot for the estimation of nitrogen, phosphorus and potassium. The samples were oven dried at 70°C temperature for 24 hours and then powdered by mechanical grinder.

(i) Nitrogen content : Modified Kjeldahl's method as described by Jackson (1973) was adopted to find out nitrogen content (%) in plant.

(ii) Phosphorus content : Phosphorus content (%) was extracted by diacid and then determined by vanadomolybdo-phosphoric acid yellow colour method as described by Jackson (1973).

(iii) Potassium content : Potassium content (%) was extracted by diacid and then determined by flame photometer method (Jackson 1973).

Nutrient uptake

$$\text{Nutrient uptake (Kg ha}^{-1}\text{)} = \frac{\text{Nutrient content (\%) in dry fodder} \times \text{Dry fodder yield (Kg ha}^{-1}\text{)}}{100}$$

Statistical analysis of the individual year data of various characters studied in the investigation was carried out as per randomised block design (Factorial). Significance of variance was tested by "F" test (Panse and Sukhatme, 1985). Pooled analysis of two years data was carried out as per method described by Steel and Torrie (1980). Summary tables for the treatment effects were prepared with Standard Error of Mean (S.Em.±) and Critical Difference (C.D.) at 5 per cent probability level for the treatments whose effects were found significant. Coefficient of Variation (C.V.) was calculated and is given in the respective tables.

RESULTS AND DISCUSSION

Effect of cutting schedule : The data in Table 1 revealed that the treatment of cutting schedule one cut (C_1) significantly recorded higher nitrogen and phosphorus content as compared

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Table 2 : Nitrogen, phosphorus and potassium uptake (Kg/ha) in dry fodder of oats as influenced by cutting schedule and nutrient management.

Treatments / Year	Nitrogen			Phosphorus			Potassium		
	2006-07	2007-08	Pooled	2006-07	2007-08	Pooled	2006-07	2007-08	Pooled
Cutting									
C_1 - One cut	152.19	139.17	145.68	18.41	18.17	18.29	80.10	91.03	85.57
C_2 - Two cuts	162.93	151.51	157.22	20.82	21.73	21.27	94.51	116.30	105.41
S.Em. +	5.03	2.76	2.87	0.46	0.42	0.31	2.17	1.82	1.41
C.D. at 5%	NS	7.85	8.07	1.32	1.18	0.87	6.18	5.18	3.98
Levels of fertilizer (RDF-80: 30: 00 N: P_2O_5: K_2O Kg ha^{-1})									
D_1 - 50 % RDF	127.56	114.85	121.20	15.36	15.95	15.65	70.79	84.29	77.54
D_2 - 75 % RDF	148.11	146.90	147.51	18.29	19.42	18.86	82.48	101.96	92.22
D_3 - 100 % RDF	173.35	157.06	165.20	21.77	21.61	21.69	95.86	110.72	103.29
D_4 - 125 % RDF	181.23	162.55	171.89	23.04	22.82	22.93	100.10	117.70	108.90
S.Em. +	7.11	3.90	4.05	0.65	0.59	0.44	3.07	2.57	2.00
C.D. at 5%	20.27	11.11	11.41	1.86	1.67	1.24	8.74	7.32	5.63
Levels of FYM									
F_0- Without FYM	142.47	132.74	137.61	18.18	18.41	18.29	81.01	93.50	87.26
F_1- 15 t ha^{-1} FYM	172.65	157.94	165.30	21.05	21.49	21.27	93.60	113.83	103.72
S.Em. +	5.03	2.76	2.87	0.46	0.42	0.31	2.17	1.82	1.41
C.D. at 5%	14.33	7.85	8.07	1.32	1.18	0.87	6.18	5.18	3.98
Interactions (C.D. at 5%)									
C x D	NS	15.71	NS	NS	NS	NS	NS	NS	NS
C x F	NS	NS	NS	NS	NS	NS	NS	7.32	NS
D x F	NS	NS	NS	NS	NS	NS	NS	NS	NS
C x D x F	NS	NS	NS	NS	NS	NS	NS	NS	NS
C.V. %	18.05	10.72	15.14	13.31	11.77	12.55	14.05	9.91	11.85

to C_2 (two cuts) during both the years and in pooled analysis. Treatment one cut (C_1) and average of two cuts (C_2) recorded 1.80 and 1.58% of nitrogen and 0.226 and 0.214% of phosphorus content, respectively in pooled results. The differences in potassium content in dry fodder of oats were not found significant due to different cutting schedule during both the years and in pooled analysis.

The treatment of cutting schedule C_2 (two cuts) produced significantly higher nitrogen uptake than one cut (C_1) during the year of 2007-08 and in pooled analysis. Treatment one cut (C_1) and total of two cuts (C_2) recorded 145.68 and 157.22 Kg ha^{-1} nitrogen uptake, respectively in pooled results. Cutting schedule C_2 (two cuts) produced significantly higher phosphorus and potassium uptake as compared to one cut (C_1) during both the years and in pooled data. Treatment one cut (C_1) and total of two cuts (C_2) recorded 18.29 and 21.27 Kg ha^{-1} phosphorus and 85.57 and 105.41 Kg ha^{-1} potassium uptake by forage oats, respectively in pooled results (Table 2).

The per cent increase in nitrogen and phosphorus contents in dry fodder under C_1 might be due to prolonged crop growth duration as in case of plant height, number of tillers and leaves per plant. More over benefit of profuse root system as more photosynthesis materials divert to root zone in one cut (C_1) instead of utilized in regrowth under C_2 (two cuts) results in large volume of soil contact for nutrient uptake by roots. While higher removal of nitrogen phosphorus and potassium by forage oats under C_2 might be due to higher dry fodder (total of first and second cut) yield. The results are in conformity with the findings of Sharma *et al.*, (2001).

Effect of inorganic fertilizers : A perusal of data in Table 1 revealed that treatment D_4 significantly recorded higher nitrogen content over rest of fertilizer levels, but it was statistically at par with D_3 during both the years and in pooled analysis, whereas, treatment D_2, D_3 and D_4 were on par during the year of 2007-08. Treatment D_4 significantly recorded higher phosphorus content over rest of fertilizer levels during both the years and in pooled analysis, however, treatment D_3 was at par during the year of 2007-08. Treatment D_4 significantly recorded higher potassium content over rest of fertilizer levels during both the years and in pooled analysis, however, treatment D_3 was at par during the year of 2006-07.

Nitrogen, phosphorus and potassium uptakes were increased progressively as the levels of inorganic fertilizer from D_1 (50 % RDF) to D_4 (125 % RDF) during the years of 2006-

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Table 3 : Interaction effect of C x D on P content in dry fodder of oats during the both the years and in pooled results .

Cutting	P content in dry fodder (%)			
	Levels of fertilizer dose			
	D_1	D_2	D_3	D_4
	2006-07			
C_1	0.192	0.213	0.238	0.251
C_2 (mean)	0.191	0.207	0.217	0.224
S.Em. ±	0.004			
C.D. at 5%	0.011			
	2007-08			
C_1	0.196	0.224	0.246	0.252
C_2 (mean)	0.204	0.210	0.223	0.234
S.Em. ±	0.006			
C.D. at 5%	0.016			
	Pooled			
C_1	0.194	0.218	0.242	0.252
C_2 (mean)	0.197	0.208	0.220	0.229
S.Em. ±	0.003			
C.D. at 5%	0.010			

Interaction effects of Cx D on N uptake by dry fodder oats during the year of 2007-08

Cutting	N uptake in dry fodder (Kg ha^{-1})			
	Levels of fertilizer dose			
	D_1	D_2	D_3	D_4
C_1	116.62	144.72	145.89	149.46
C_2 (mean)	113.08	149.08	168.23	175.64
S.Em. +	5.51			
C.D. at 5%	15.71			

Table 4 : Interaction effects of C x F on K uptake by dry fodder oats during the year of 2007-08.

Cutting	K uptake by dry fodder (Kg ha^{-1})	
	Levels of fertilizer dose	
	F_0	F_1
C_1	83.84	98.22
C_2 (mean)	103.15	129.45
S.Em. ±	2.57	
C.D. at 5%	7.32	

07, 2007-08 and in pooled analysis. Treatment D_4 significantly recorded higher nitrogen, phosphorus and potassium uptake over rest of fertilizer levels, however, treatment D_3 was at par during both the years and in pooled analysis (Table 2).

The increase in content with increase in inorganic fertilizer levels might be due to greater availability of nutrients as results of better growth parameters and the beneficial effect of starter dose of fertilizers result in profuse root system which makes efficient uptake and utilization of applied as well as native soil nutrients. The levels of inorganic fertilizers brought out significant differences in total nitrogen, phosphorus and potassium uptake during both the years and in pooled results. Data in Table 1 indicated an increase in removal of nitrogen, phosphorus and potassium uptakes to the extent of 41.82, 46.52 and 40.44 per cent under 125 % RDF, respectively as compared to 50 % RDF in pooled results. This could be ascribed to respective higher nutrients content and dry matter yields at higher level of inorganic fertilizers. Similar results have also been reported by Vyas (1986) that application of fertilizer, markedly, enhanced the uptake of nutrients in oats, Sharma, *et al.*, (2001) also observed that the application of N up to 100 Kg ha^{-1} significantly increased N and P contents, where as, successive increment in P levels up to 60 Kg ha^{-1} increased N and P contents and uptakes.

Effect of FYM (organic manure) : The data given in Table 1 revealed that application of 15 t FYM ha^{-1} significantly recorded the highest nitrogen and phosphorus contents in both years and in pooled analysis. The application of 15 t FYM ha^{-1} significantly recorded the highest nitrogen, phosphorus and potassium uptakes in both years and in pooled analysis (Table 2).

Higher removal of nitrogen, phosphorus and potassium by forage oats under F_1 might be due to higher dry fodder yield and higher content in dry fodder of oats. The percentage increase in nitrogen and phosphorus content in dry fodder under treatment F_1 might be outcome of increased availability of nitrogen and phosphorus to the plant upon decomposition of applied FYM and due to similar cause of proliferation of roots and favouarble soil physical environment and addition of organic manure results in large volume of soil contact for nutrient absorption. These results are in agreement with the findings by Singh and Dahiya (1980) that phosphorus uptake increased with application of FYM over control (with out FYM).

Interaction effect : The data in Table 3 revealed that phosphorus content in forage oats was recorded significantly higher in treatment combination C_1D_4 during the years of 2006-07, 2007-08 and in pooled results, but it was statistically remained at par with treatment combination C_1D_3 during the year of 2007-08 and in pooled results. Interaction effect of different cutting schedule and inorganic fertilizer levels (C x D) on nitrogen uptake was found significant during the year of 2007-08 (Table 3). Treatment combination C_2D_4 recorded significantly higher (175.64 Kg ha^{-1}) nitrogen uptake by forage oats, which was at par with treatment combination C_2D_3 during the year of 2007-08. The interaction effect between cutting schedule and level of inorganic fertilizers might be due to higher nutritional requirement under two cuts than one cut schedule coincides with application of higher dose of inorganic fertilizer which supplies greater quantities of nitrogen and phosphorus with each incremental level to meet demand of intensive cutting schedule. Adequate supply of major nutrient like N and P results in better utilization of native soil nutrients, which results in better content as well as uptake by developed and profuse root system of forage oats.

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Table 2 : Nitrogen, phosphorus and potassium uptake (Kg/ha) in dry fodder of oats as influenced by cutting schedule and nutrient management.

Treatments / Year	Nitrogen			Phosphorus			Potassium		
	2006-07	2007-08	Pooled	2006-07	2007-08	Pooled	2006-07	2007-08	Pooled
Cutting									
C_1 - One cut	152.19	139.17	145.68	18.41	18.17	18.29	80.10	91.03	85.57
C_2 - Two cuts	162.93	151.51	157.22	20.82	21.73	21.27	94.51	116.30	105.41
S.Em. +	5.03	2.76	2.87	0.46	0.42	0.31	2.17	1.82	1.41
C.D. at 5%	NS	7.85	8.07	1.32	1.18	0.87	6.18	5.18	3.98
Levels of fertilizer (RDF-80: 30: 00 N: P_2O_5: K_2O Kg ha^{-1})									
D_1 - 50 % RDF	127.56	114.85	121.20	15.36	15.95	15.65	70.79	84.29	77.54
D_2 - 75 % RDF	148.11	146.90	147.51	18.29	19.42	18.86	82.48	101.96	92.22
D_3 - 100 % RDF	173.35	157.06	165.20	21.77	21.61	21.69	95.86	110.72	103.29
D_4 - 125 % RDF	181.23	162.55	171.89	23.04	22.82	22.93	100.10	117.70	108.90
S.Em. +	7.11	3.90	4.05	0.65	0.59	0.44	3.07	2.57	2.00
C.D. at 5%	20.27	11.11	11.41	1.86	1.67	1.24	8.74	7.32	5.63
Levels of FYM									
F_0 - Without FYM	142.47	132.74	137.61	18.18	18.41	18.29	81.01	93.50	87.26
F_1 - 15 t ha^{-1} FYM	172.65	157.94	165.30	21.05	21.49	21.27	93.60	113.83	103.72
S.Em. +	5.03	2.76	2.87	0.46	0.42	0.31	2.17	1.82	1.41
C.D. at 5%	14.33	7.85	8.07	1.32	1.18	0.87	6.18	5.18	3.98
Interactions (C.D. at 5%)									
C x D	NS	15.71	NS	NS	NS	NS	NS	NS	NS
C x F	NS	NS	NS	NS	NS	NS	NS	7.32	NS
D x F	NS	NS	NS	NS	NS	NS	NS	NS	NS
C x D x F	NS	NS	NS	NS	NS	NS	NS	NS	NS
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to C_2 (two cuts) during both the years and in pooled analysis. Treatment one cut (C_1) and average of two cuts (C_2) recorded 1.80 and 1.58% of nitrogen and 0.226 and 0.214% of phosphorus content, respectively in pooled results. The differences in potassium content in dry fodder of oats were not found significant due to different cutting schedule during both the years and in pooled analysis.

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Cutting	N uptake in dry fodder (Kg ha^{-1})			
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The results revealed that interaction effect of different cutting schedule and organic fertilizer levels (C x F) was found significant on potassium uptake by forage oats during the year of 2007-08 (Table 4). The treatment combination C_2F_1 significantly recorded the highest (129.45 Kg ha^{-1}) potassium uptake as compared to rest of the treatment combinations during the year of 2007-08. This might be due to improvement in soil physical environment with addition of FYM, which results in better uptake of native soil potassium and higher dry fodder yield.

CONCLUSION

From for going experimental results, it can be concluded that contents of nitrogen and phosphorus were significantly recorded higher under one cutting schedules, 125% RDF and application of 15 t FYM/ha during both the years and in pooled results, where as potassium content was significantly recorded higher under 125% RDF and application of 15 t FYM/ha during both the years. The uptakes of nitrogen, phosphorus and potassium were significantly recorded higher under two cutting schedules, 125% RDF and application of 15 t FYM/ha during both the years and in pooled results.

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Studies on the Effect of Micronutrients on Fodder Yield and Quality in Perennial Fodder Crops

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Field experiment was conducted to study the effect of different micronutrients (Iron, Zinc, Copper, Manganese & Boron) applied through soil and foliar application with and without farm yard manure on fodder yield and quality in perennial fodder crops. The results revealed that application of FYM + recommended dose of NPK + foliar spray of micronutrients registered the maximum green fodder yield of 322.6, 237.5, 214.2, 145.4 and 146.1 t ha^{-1} yr^{-1} respectively in cumbu napier hybrid, guinea grass, sorghum, lucerne and hedge lucerne respectively. There was no significant improvement in fodder quality due to application of micronutrients.

Keywords : Micronutrients, Soil application, Foliar spray, Fodder yield, Fodder quality.

INTRODUCTION

Micronutrient deficiency is a massive global problem afflicting 3.0 billion people worldwide, mostly women, infants and children, of which one third live in India. Iron (Fe), Zinc (Zn), vitamin A (beta-carotene), Selenium (Se) and Iodine (I) are the most prevalent trace-nutrient deficiencies. A major means of tackling the increasing micronutrient malnutrition problem in the poor urban and rural population in India is through a major focus on enriching food grains with essential micronutrients, through breeding and /or appropriate management practices. Intensive cropping over the period has accentuated extensive micronutrient deficiencies in soils and crops and so the micronutrient content in seed and fodder is declining by about 1.0 to 1.5 times in major cereal and pulse crops. To increase the micronutrients content in grains and fodder, a clear scientific understanding of mechanisms of micronutrient mobilization, absorption, translocation and re translocation in seed-soil-plant-seed-animal/human chain is essential.

Brown *et al.* (1989) reported for manganese (Mn) deficiency in wheat decreasing lignin content while phenols and flavonoids accumulated. Shkolnik (1984) related an increased content of phenols to boron (B) deficiency. With the light above, the present investigation was taken up to assess the micronutrient requirement of perennial fodder crops to maximize the fodder yield and to improve fodder quality which in turn overcome the problem of micronutrient mal nutrition in animals.

MATERIAL AND METHODS

A field experiment was conducted at New Area Farm of Department of Forage Crops, TNAU, Coimbatore to study the effect of different micronutrients as soil application and foliar spray on fodder yield and quality in perennial fodder crops *viz.*, cumbu napier hybrid, guinea grass, multi-cut fodder sorghum COFS 29, lucerne and hedge lucerne. The soil of the experimental plot was sandy clay loam in texture and neutral in reaction (pH 7.94, EC 0.20 dSm^{-1}),low in available N (alkaline permanganate extractable,233 Kg ha^{-1}), medium in available P (0.5 N $NaHCO_3$ extractable, 22 Kg ha^{-1}) and high in available K (NH_4 OAC extractable, 640 Kg ha^{-1}). The micro nutrient status of the soil was as follows: DTPA extractable zinc 4.20 ppm, copper 2.89 ppm, iron 7.96 and manganese 8.44 ppm. Recommended dose of NPK fertilizers (Cumbu napier hybrid -150:50:40 Kg NPK ha^{-1} ; guinea grass - 150:40:40 Kg NPK ha^{-1}; Sorghum - 90:40:40 Kg NPK ha^{-1}; Lucerne - 80:120:20 Kg NPK ha^{-1} and hedge Lucerne - 80:120:20 Kg NPK ha^{-1}) were applied to all fodder crops. The treatments were compared at two FYM levels (With FYM - 25 t ha^{-1} and Without FYM). The experiment was conducted in split split plot design having perennial fodder crops as main plot treatments, FYM levels as sub plot treatments and micronutrient treatments as sub sub plot treatments(NPK alone, NPK + $FeSO_4$ 50 Kg ha^{-1}, NPK + $FeSO_4$ 50 Kg ha^{-1} + $ZnSO_4$ 25 Kg ha^{-1}, NPK+ $FeSO_4$ 50 Kg ha^{-1}+ $ZnSO_4$ 25 Kg ha^{-1} + $MnSO_4$ 10 Kg ha^{-1}, NPK + $FeSO_4$ 50 Kg ha^{-1} + $ZnSO_4$ 25 Kg ha^{-1} + $MnSO_4$ 10 Kg ha^{-1} + $CuSO_4$ 5Kg ha^{-1}, NPK + $FeSO_4$ 50 Kg ha^{-1} + $ZnSO_4$ 25 Kg ha^{-1} + $MnSO_4$ 10 Kg ha^{-1} + $CuSO_4$ 5 Kg ha^{-1} + Borax 2 Kg ha^{-1}, Foliar spray of micronutrients at recommended dose (Fe 1 %, Zn 0.5%, Cu 0.5%, Mn 0.5% and Borax 0.2 %) with two replications.

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Table 1 : Effect of micronutrient treatments on green and dry fodder yields (t ha^{-1} yr^{-1}).

S. No.	Treatments	Cumbu napier hybrid			Guinea grass			Sorghum COFS 29			Lucerne			Hedge Lucerne		
		+FYM (25tha^{-1})	–FYM	Mean	+FYM (25tha^{-1})	–FYM	Mean	+FYM (25tha^{-1})	–FYM	Mean	+FYM (25tha^{-1})	–FYM	Mean	+FYM (25tha^{-1})	–FYM	Mean
1.	NPK alone	243.8 (53.6)	225.6 (49.6)	234.7 (51.6)	145.6 (29.1)	127.3 (25.5)	136.5 (27.3)	133.2 (31.4)	117.6 (27.8)	125.4 (29.6)	71.6 (20.2)	59.2 (16.6)	65.4 (18.3)	70.2 (18.3)	53.7 (13.4)	62.0 (15.9)
2.	NPK+ $FeSO_4$ 50 Kg/ha	256.5 (56.4)	238.2 (52.4)	247.4 (54.4)	160.4 (32.1)	142.1 (28.4)	151.3 (30.3)	146.7 (34.6)	128.4 (30.3)	137.6 (32.5)	83.9 (23.5)	71.5 (20.0)	77.7 (21.8)	82.7 (21.5)	66.2 (17.2)	74.5 (19.4)
3.	NPK+ $FeSO_4$ 50 Kg/ha + $ZnSO_4$ 25 Kg/ha	269.1 (59.2)	250.8 (55.2)	260.0 (57.2)	173.2 (34.6)	154.8 (31.0)	164.0 (32.8)	160.2 (37.8)	141.9 (33.5)	151.1 (35.7)	96.2 (26.9)	83.8 (23.5)	90.0 (25.2)	95.2 (24.8)	78.7 (20.5)	87.0 (22.7)
4.	NPK+ $FeSO_4$ 50 Kg/ha + $ZnSO_4$ 25 Kg/ha + $MnSO_4$ 10 Kg/ha	283.2 (62.3)	265.6 (58.4)	274.4 (60.4)	190.0 (38.0)	169.7 (33.9)	179.9 (36.0)	173.7 (41.0)	155.4 (36.7)	164.6 (38.9)	108.5 (30.4)	96.1 (26.9)	102.3 (28.7)	107.2 (27.9)	90.7 (23.6)	99.0 (25.8)
5.	NPK+ $FeSO_4$ 50 Kg/ha + $ZnSO_4$ 25 Kg/ha + $MnSO_4$ 10 Kg/ha + $CuSO_4$ 5 Kg/ha	294.7 (64.8)	277.8 (61.1)	286.3 (63.0)	204.8 (41.0)	184.3 (36.9)	194.6 (39.0)	180.2 (44.2)	168.9 (39.9)	178.1 (42.1)	120.8 (33.8)	108.4 (30.4)	114.6 (32.1)	121.5 (31.6)	105.0 (27.3)	113.3 (29.5)
6.	NPK+ $FeSO_4$ 50 Kg/ha + $ZnSO_4$ 25 Kg/ha + $MnSO_4$ 10 Kg/ha + $CuSO_4$ 5 Kg/ha + Borax 2 Kg/ha	307.3 (67.6)	293.2 (64.5)	300.3 (66.1)	218.3 (43.7)	200.3 (40.1)	209.3 (41.9)	200.7 (47.4)	182.4 (43.0)	191.6 (45.2)	133.1 (37.3)	120.7 (33.8)	126.9 (35.6)	133.9 (34.8)	117.4 (30.5)	125.7 (32.7)
7.	NPK + Foliar spray	322.6 (71.0)	305.2 (67.1)	313.9 (69.1)	237.5 (47.5)	217.3 (43.5)	227.4 (45.5)	214.2 (50.6)	195.9 (46.2)	205.1 (48.4)	145.4 (40.7)	133.0 (37.2)	139.2 (39.0)	146.1 (38.0)	129.6 (33.7)	137.9 (35.9)
	Mean	282.5 (62.1)	265.2 (58.3)		190.0 (38.0)	170.8 (34.2)		173.7 (41.0)	155.8 (36.8)		108.5 (30.4)	96.1 (26.9)		108.1 (28.1)	91.6 (23.7)	
		SED	CD (0.05)		SED	CD (0.05)		SED	CD (0.05)		SED	CD (0.05)		SED	CD (0.05)	
	M	24.7 (5.8)	12.1 (2.9)		23.1 (4.2)	11.2 (2.1)		23.8 (5.6)	11.9 (2.8)		19.8 (4.4)	9.9 (2.2)		20.5 (4.2)	10.2 (2.1)	
	S	20.9 (5.1)	10.2 (2.5)		19.3 (2.8)	9.6 (1.9)		21.4 (4.2)	10.7 (2.1)		17.5 (3.8)	8.7 (1.9)		19.8 (3.6)	9.8 (1.8)	
	M @ S	28.6 (6.6)	14.2 (3.3)		26.9 (5.8)	13.1 (2.9)		25.6 (6.2)	12.8 (3.1)		21.8 (5.4)	10.9 (2.7)		22.5 (5.6)	11.2 (2.8)	
	S @ M	27.9 (4.3)	13.9 (2.1)		25.7 (4.4)	12.8 (2.2)		22.2 (5.1)	11.1 (2.6)		20.3 (4.2)	10.1 (2.1)		20.6 (4.4)	10.3 (2.2)	

Figures in ( ) indicates dry fodder yield

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Table 2 : Effect of micronutrient treatments on crude protein yield (t ha^{-1} yr^{-1}).

S. No.	Treatments	Cumbu napier hybrid			Guinea grass			Sorghum COFS 29			Lucerne			Hedge Lucerne		
		+FYM (25tha^{-1})	–FYM	Mean	+FYM (25tha^{-1})	–FYM	Mean	+FYM (25tha^{-1})	–FYM	Mean	+FYM (25tha^{-1})	–FYM	Mean	+FYM (25tha^{-1})	–FYM	Mean
1.	NPK alone	5.63	5.21	5.42	2.04	1.79	1.92	2.65	2.34	2.50	3.98	3.30	3.64	3.51	2.57	3.04
2.	NPK+ $FeSO_4$ 50 Kg/ha	5.92	5.50	5.71	2.25	1.99	2.12	2.92	2.55	2.74	4.68	3.98	4.33	4.13	3.30	3.72
3.	NPK+ $FeSO_4$ 50 Kg/ha + $ZnSO_4$ 25 Kg/ha	6.22	5.80	6.01	2.43	2.17	2.30	3.19	2.82	3.01	5.35	4.68	5.02	4.76	3.94	4.35
4.	NPK+ $FeSO_4$ 50 Kg/ha + $ZnSO_4$ 25 Kg/ha + $MnSO_4$ 10 Kg/ha	6.54	6.13	6.34	2.66	2.38	2.52	3.46	3.09	3.28	6.05	5.35	5.70	5.36	4.53	4.95
5.	NPK+ $FeSO_4$ 50 Kg/ha + $ZnSO_4$ 25 Kg/ha + $MnSO_4$ 10 Kg/ha + $CuSO_4$ 5 Kg/ha	6.80	6.42	6.61	2.87	2.59	2.73	3.73	3.36	3.55	6.73	6.05	6.39	6.07	5.24	5.66
6.	NPK+ $FeSO_4$ 50 Kg/ha + $ZnSO_4$ 25 Kg/ha + $MnSO_4$ 10 Kg/ha + $CuSO_4$ 5 Kg/ha + Borax 2 Kg/ha	7.10	6.77	6.94	3.06	2.81	2.94	4.00	3.62	3.81	7.42	6.73	7.08	6.68	5.86	6.27
7.	NPK + Foliar spray	7.46	7.05	7.26	3.33	3.05	3.19	4.27	3.89	4.08	8.10	7.40	7.75	7.30	6.47	6.89
	Mean	6.52	6.13		2.66	2.40		3.46	3.10		6.04	5.36		5.40	4.56	
		SED	CD (0.05)		SED	CD (0.05)		SED	CD (0.05)		SED	CD (0.05)		SED	CD (0.05)	
	M	0.62	0.31		0.56	0.28		0.60	0.30		0.56	0.28		0.62	0.31	
	S	0.56	0.28		0.44	0.22		0.54	0.27		0.52	0.26		0.54	0.27	
	M @ S	0.44	0.22		0.50	0.25		0.42	0.21		0.62	0.31		0.64	0.32	
	S @ M	0.41	0.21		0.48	0.24		0.38	0.19		0.58	0.29		0.58	0.29	

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Seven cuttings were harvested in all fodder crops except in sorghum COFS 29 (5 cuttings were harvested). At each harvest in all fodder crops, green fodder yield was recorded. Plant samples were collected at each harvest and analysed for crude protein content. Dry fodder yield was computed by using green fodder yield and dry matter content. Crude protein yield was calculated by multiplying crude protein content with dry fodder yield.

RESULTS AND DISCUSSION

Green Fodder Yield : In cumbu napier hybrid, combined application of micronutrients in soil registered the green fodder yield of 307.3 t ha^{-1} yr^{-1} in FYM @ 25 t ha^{-1} applied plots whereas the foliar application at recommended dose recorded the maximum green fodder yield of 322.6 t ha^{-1} yr^{-1}. Maximum dry fodder yield (71.0 t ha^{-1} yr^{-1}) was recorded in foliar spray of micronutrients followed by soil application along with FYM in cumbu napier hybrid. In sorghum COFS 29, maximum dry fodder yield of 50.6 t ha^{-1} yr^{-1} was recorded in foliar spray of micronutrients followed by soil application (47.4 t ha^{-1} yr^{-1}) (Table 1). Ashoka *et al.* (2008) indicated that yield of baby corn and fodder yield were significantly higher in the treatments applied with recommended NPK + 25 Kg $ZnSO_4$ +10 Kg $FeSO_4$ + 35 Kg vermicompost. Abundant supply of nutrients through micronutrients with inorganics and recommended dose of fertilizers might have increased the proto plasmic constituents and accelerated the process of cell division and cell elongation. This in turn resulted in increased fodder yield. Similar findings were reported by Auti *et al.* (1999). Similar trend of results were observed in guinea grass, sorghum COFS 29, lucerne and hedge lucerne.

Crude Protein Yield : The results revealed that in cumbu napier hybrid, combined application of micronutrients through foliar spray in FYM treatment registered the higher crude protein yield of 7.46 t ha^{-1} yr^{-1} whereas soil application recorded 7.10 t ha^{-1} yr^{-1}. In sorghum COFS 29 and guinea grass, foliar spray of micronutrients recorded the maximum crude protein yield of 4.27 t ha^{-1} yr^{-1} and 4.00 t ha^{-1} yr^{-1} respectively followed by soil application (Table 2). The increase in crude protein yield was mainly due to the increase in green and dry fodder yields which in turn due to micronutrient application. Similar findings were reported by Khalid Mahmud *et al.* (2003).

From the study, it was concluded that combined application of micronutrients through foliar spray along with recommended dose of NPK and FYM 25 t ha^{-1} is to be recommended to obtain maximum green fodder, dry fodder and crude protein yields in perennial fodder crops.

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Effect of Different Supplements on Mineral Element Content of *Pleurotus* species

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The species *Pleurotus sajor-caju* and *Pleurotus florida* were cultivated on paddy straw beds with and without supplements *viz*, wheat bran, rice bran, gram flour, neem cake and inorganic fertilizers (Plate I). The paddy straw (1 Kg dry or about 3.5 Kg wet weight) and spawn @ 3% wet weight of paddy straw were common to all the treatments. The supplements were added on dry weight basis. One gram digested sample was used for estimation of phosphorus, potassium, calcium sulphur and iron content. The results of calcium and iron were confirmed on Perkin Elmer's AAnalyst 200 model of Atomic Absorption Spectrophotometer (AAS). The data on nutritionally important element content revealed that the oven dried mushroom contained 1.58 to 2.42 per cent phosphorus, 2,30 to 3.18 per cent potassium, 22.7 to 44.0 mg calcium, 178.8 to 257.9 mg sulphur and 11.1 to 15.4 mg iron per 100 g of mushroom.

Keywords : AAS, mineral element, *Pleurotus florida, Pleurotus sajor-caju,* Supplements, Paddy straw.

INTRODUCTION

Mushrooms are well known as a good source of dietary proteins. There are several reports regarding the protein, amino acid and carbohydrate contents of mushrooms (Bano *et al.* 1971; Jandaik and Kapoor, 1975) but the information regarding mineral constituents are rather meager. The present paper deals with the mineral element analysis of *Pleurotus sajor-caju* and *Pleurotus florida*. Mushroom samples were analysed for Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Sulphur (S) and Iron (Fe) contents in their sporophores.

MATERIAL AND METHODS

The *Pleurotus* species were cultivated on paddy straw beds prepared with and without supplements in a room previously disinfected with 2 per cent formalin. The paddy straw (1 Kg dry weight) was chopped into pieces of 5 to 8 cm length, filled in gunny bag and soaked with tap water for 12 hours. After soaking, excess water was drained off and pasteurized by dipping in hot water at 82°C for one hour. The supplements *viz.*, wheat bran, rice bran, gram flour, neem cake and inorganic fertilizers were sterilized in autoclave at 1 Kg/cm² pressure for one hour. The polypropylene bags (35 cm x 55 cm) were sterilized by dipping them in 2 per cent formalin solution and filled with pasteurized wet paddy straw (approx. 3.5 Kg wet weight), spawn and supplements. The spawn and supplements were added along the periphery of the bed in five layers as depicted in Figure 1.

The paddy straw (1 Kg dry or about 3.5 Kg wet weight) and spawn @ 3% wet weight of paddy straw were common to all the treatments. The supplements were added on dry weight basis as per the treatment details given below.

Table 1. Treatment details.

Treatment	Description
T_1	Control
T_2	10% Rice bran
T_3	10% Wheat bran
T_4	5% Gram flour
T_5	20% Neem cake
T_6	Fertilizer mixture (Urea 1.15g + KH_2PO_4 4.38 g + Cellulose powder 94.47 g)

The treatments were replicated three times and were same for the species *viz.*, *Pleurotus sajor-caju* and *Pleurotus florida*. The treatment details are given in Table 1. The experiment was started on 24th September 2005 in Department of Agricultural Chemistry and Soil Science, College of Agriculture, Dapoli (MS) under standard recommended conditions.

The sporophores after harvest were dried in oven at 70°C temperature until complete drying. The dried mushrooms were ground to powder in a waring blender. This powder was

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used for analysis of P, K, Ca, S and Fe. Oven dried mushroom powder (0.5 g) was digested in Pelican's Kjel plus IL8M12 digestion unit with minimum volume (5 ml) of concentrated H_2SO_4 using catalyst mixture. The volume of digest was made to 50 ml and an aliquot of 5ml digested sample was used for estimation of nitrogen by micro-kjeldahl method (Johari *et al,* 2000) using Pelican's DISTIL-M distillation unit.

One gram sample was digested with 10 ml of conc. HNO_3 and 5 ml 70 per cent $HClO_4$. After complete digestion, the volume was made to 50 ml. This diacid extract was used for estimation of phosphorus, potassium, calcium and sulphur by using standard methods.

RESULTS AND DISCUSSION

The data on effect of different supplements on content of nutritionally important elements in *Pleurotus* species (Table 2) revealed that the phosphorus content ranged from 1.58 to 2.42 per cent with a mean value of 2.03 per cent on dry weight basis. The fertilizer treatment (T_6) produced the highest amount of phosphorus (2.31%) in *Pleurotus* species which was significantly higher than all other treatments except gram flour treatment (T_4) which was at par with it. The effect due to species was significant. The *Pleurotus florida* recorded significantly higher phosphorus content (2.09%) as compared with *Pleurotus sajor-caju* (1.97%). The interaction effect of species and treatment revealed that the phosphorus content on dry weight basis increased significantly in all the treatments and the increase was maximum and significant (2.42%) in neem cake-*Pleurotus florida* (V_2T_5) combination followed by fertilizer-*Pleurotus sajor-caju* (V_1T_6) treatment combination. The phosphorus content values observed in present study agreed well with those (1.08 to 2.08%) reported by Sivaprakasam (1983) and Rathore and Thakore (2004). Dursun *et al.* (2006) have reported the phosphorus content in different varieties of mushroom which ranged from 0.21 to 0.28 per cent. The values observed in the present investigation fall within the range given by them. However, the phosphorus content values (446 to 882 mg/100 g) reported by Al-Kaisey *et al.* (1998), Pathak *et al.* (1998), Caglarlrmak *et al.* (2000) and Bande (2004) were lower than the phosphorus content values found in present investigation. Results as obtained in present study are similar with results reported by other workers (Anonymous, 1972; Mattila *et al.* 2001 and Mandhare *et al.* 2003).

The potassium content of *Pleurotus sajor-caju* and *Pleurotus florida* ranged from 2.30 to 3.18 per cent with a mean value of 2.75 per cent on dry weight basis. The treatment effect on potassium content of *Pleurotus* species was significant. The fertilizer treatment (T_6) produced the highest amount of potassium content (3.17%) which was significantly higher than all other treatments. All other treatments have also increased the potassium content significantly as compared with control. It was interesting to note that the significant effect of treatments on potassium content was observed in the reverse order *i.e.*, $T_6>T_5>T_4>T_3>T_2>T_1$. The effect due to species was not significant. These differences among different treatment-species combinations were, however, statistically not significant. The potassium content values (2620 to 3640 mg/100 g) reported by Sivaprakasam (1983); Gupta (1998) and Mandhare *et al.* (2003) for *Pleurotus sajor-caju* were in close agreement with potassium values obtained in present study. Mandhare *et al.* (2003) also reported potassium content value of 2400 mg per 100 g for *Pleurotus florida* which was in accordance with the potassium content value observed in present investigation. However, the potassium content values (3793 to 4730 mg/100 g) reported by Anonymous (1972), Hadwan *et al.* (1993); Mattila *et al.* (2004) and Rathore and Thakore (2004) were about 1.5 times higher than the potassium content values found in present study.

The calcium content of *Pleurotus* species ranged from 22.7 to 44.0 mg per 100 g with a mean value of 36.7 mg per 100 g on dry weight basis. The highest amount of calcium (43.3 mg/100 g) was produced by fertilizer treatment (T_6) in *Pleurotus* species which was significantly higher than all other treatments except wheat bran (T_3) and neem cake (T_5) treatments which were statistically at par. All other treatments have also increased the calcium content significantly as compared with control. The effect due to species was non-significant. The interaction of species with treatment revealed that the calcium content was increased significantly and the increase was maximum and significant (44.0 mg/100 g) in fertilizer - *Pleurotus florida* treatment combination (V_2T_6) and neem cake - *Pleurotus florida* combination (V_2T_5). The calcium content values (23.6 to 39.6 mg/100 g) of mushroom reported by Anonymous (1972), Pathak *et al.* (1998), Caglarlrmak *et al.* (2001) and Bande (2004) were in close agreement with calcium content values obtained in the present investigation. However, the calcium content values (80 to 150 mg/100 g) reported by Hadwan *et al.* (1993), Singh *et al.* and Gupta (1998) were 2 to 3 times higher than the calcium content values found in present study.

The sulphur content of *Pleurotus* species ranged from 178.8 to 257.9 mg per 100 g with a mean value of 218.1 mg per 100 g. The treatment effect in respect of sulphur content was significant. The sulphur content was decreased significantly due to different treatments except in wheat bran (T_3) and fertilizer treatment (T_6) which were statistically at par with control. The differences due to species were non-significant. The interaction of species with treatments revealed that the sulphur content was significantly affected by treatment-species interaction. The highest amount of sulphur (257.9 mg/100 g) was recorded in *Pleurotus florida* - control (V_2T_1) followed by *Pleurotus florida* -fertilizer combination. The sulphur content values (222 to 237 mg/100 g) reported by Bande (2004) in *Pleurotus* species was in close agreement with the sulphur content values observed in present investigation. While, the sulphur content value (106 mg/100 g) reported by Anonymous (1972) was lower than the sulphur content values obtained in present study. However, the sulphur content value (310 mg/100 g) found by Singh *et al.* (1996) was much higher than the sulphur content value found in present investigation.

The iron content of oyster mushroom ranged from 11.1 to 15.4 mg per 100 g with a mean value of 12.7 mg per 100 g on dry weight basis. The highest amount of iron (13.9 mg/100 g) was produced by gram flour treatment (T_4) in *Pleurotus*

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Table 2 : Effect of different supplements on mineral content of Pleurotus species.

Treatment	P (%)			K (%)			Ca (mg/100 g)			S (mg/100 g)			Fe (mg/100 g)		
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1-Control	1.58	1.67	1.62	2.30	2.35	2.33	30.7	22.7	26.7	205.4	257.9	231.7	12.5	12.1	12.3
T_2-Rice bran	1.81	1.75	1.78	2.50	2.55	2.53	40.0	30.7	35.3	178.8	211.7	195.3	13.4	13.6	13.5
T_3-Wheat bran	2.08	2.20	2.14	2.60	2.79	2.69	38.7	41.3	40.0	226.4	228.1	227.2	11.1	12.4	11.7
T_4-Gram flour	2.19	2.32	2.25	2.81	2.88	2.84	32.0	37.3	34.7	233.0	198.6	215.8	12.5	15.4	13.9
T_5-Neem cake	1.78	2.42	2.10	2.97	2.95	2.96	36.0	44.0	40.0	230.7	188.6	209.6	12.7	11.6	12.2
T_6-Fertilizer	2.41	2.21	2.31	3.17	3.18	3.17	42.7	44.0	43.3	223.5	235.2	229.3	12.1	13.0	12.5
Mean	**1.97**	**2.09**	**2.03**	**2.73**	**2.78**	**2.75**	**36.7**	**36.7**	**36.7**	**216.3**	**220.0**	**218.1**	**12.4**	**13.0**	**12.7**
S.E. ± (T)		0.01			0.03			1.18			2.39			0.30	
C.D. @ 1%		**0.06**			**0.10**			**4.70**			**9.50**			**1.20**	
S.E. ± (V)		0.08			0.01			0.68			1.38			0.17	
C.D. @ 1%		**0.03**			**N.S.**			**N.S.**			**N.S.**			**N.S.**	
S.E. ± (V x T)		0.02			0.04			1.67			3.37			0.43	
C.D. @ 1%		**0.08**			**N.S.**			**6.65**			**13.44**			**1.70**	

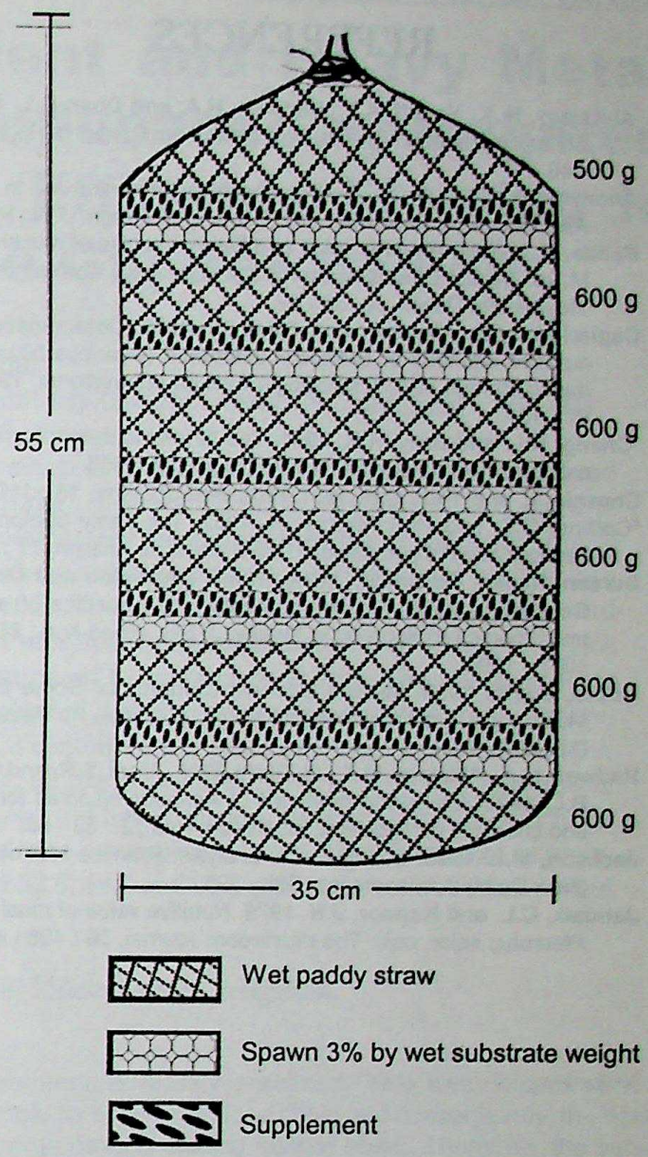

Fig. 1. Mushroom bed

species which was significantly higher than all other treatments except rice bran treatment (T_2) which was at par. All other treatments were statistically at par with their control in respect to iron content. The effect of iron content due to species was not significant while, the interaction effect was significant. The gram flour treatment produced the highest amount of iron (15.4 mg/100 g) in *Pleurotus florida* (V_2) which was significantly higher than all other treatment-species combinations.

The iron content values (8.8 to 15.2 mg/100 g) reported (Anonymous 1972) are in close agreement with the value in present study. Similar findings were reported by Singh *et al.* (1996), Kannan *et al.* (1998), Caglarlrmak *et al.* (2001) and Bande (2004) which were in accordance with the values in present investigation. The iron content values reported by Sivaprakasam (1983) and Hadwan *et al.* (1993) were however; lower than the values in present study.

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Accumulation Nutrient and Heavy Metal Content of Rice as Influenced by Irrigated with Mix Industrial Effluents Flowing in *Khari* River of Middle Gujarat Region

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A study was conducted to determine the chemical composition of rice irrigated with effluents contaminated water flowing in *Khari* river of middle Gujarat region (India). Twenty - Twenty samples of rice plant at harvest were collected from both contaminated and uncontaminated location of the area of *Nawagam-Vatava* region of Gujarat (India) where effluent canal is passed. The plant samples of grain, straw and husk of rice were used for analysis of total P, K, S, trace and heavy metals. The analytical results of rice grain, straw and husk samples indicated that the P, K and S were higher in samples of uncontaminated area except K in rice straw and husk were higher in uncontaminated area as compared to contaminated area. The micronutrients content were higher in rice grain, straw and husk of contaminated area than in uncontaminated area. Further, it was noted that among the different micronutrients, the Cu content in rice grain and husk was 40 and 48 per cent higher than samples of uncontaminated area. Further, all the heavy metals were more concentrated in rice grain, straw and husk parts in contaminated area. Among the heavy metals, the contamination of Pb, Cr and Ni was found quite higher in all three components of rice in contaminated area. The Cr content of grain, straw and husk of rice in contaminated area were higher by 52.8, 44.7 and 91.8 per cent over uncontaminated area.

Keywords : Accumulation, Heavy metals, Nutrients, Rice, Effluent water, Irrigation.

INTRODUCTION

In different parts of the country, the menace of a rapidly increasing population, the want on growth of industries and increasing urbanization has created major problems with the disposal of sewage and industrial effluents. These industries generate huge quantity of solid and liquid wastes. They contain appreciable amounts of metals besides beneficial nutrients. Therefore, their continuous application to soil may lead to accumulation of heavy metals which are likely to pose serious threat on soil health and plant growth as they depress the yield and quality. In Nawagam area villages' ponds are receiving effluent water through *Khari* canal/channel, which carries industrial effluents discharged from the industrial area of *Naroda* and *Vatava*. These effluents may or may not be biodegradable. It was also observed that there were approximately 1600 units of which, about 525 units generate effluents. Therefore, there are possibilities of the contamination of surface and ground water and soils of the area by chromium present in the wastewater released by the industry. Farmers in Nawagam area use effluent diluted with fresh canal water for irrigation purpose as and when required. In addition, water tube well of nearby effluent canal is contaminated and being used by the farmers for irrigating their fields where rice- wheat sequence is mainly practiced. This has caused serious problems concerning food chain and consequently, the health of organisms, including human being. Therefore, the survey work was carried out to investigate the heavy metal toxicity in rice crop growing in *Nawagam-Vatava* area.

MATERIAL AND METHODS

The twenty-twenty plant samples of rice were collected in the end of *Rabi* seasons from uncontaminated areas of villages like *Shrijipura* (2), *Chitrasar* (2), *Dharoda* (2), *Kathwada* (1), *Chalindra* (1), Bareja (2), Bherai (1), Bakodara jara (1), Vadala (4), Dhathal (1) and Nayaka (3) as well contaminated areas of villages like Chalinra (1), Pinglaj (2), Nawagam (3), Pansholi (2), Malarpur (1), Kanera (1), Girmatha (1), Lambha (1), Sarasa (1), Lali (2), Umiyapura (2), Bidaj (1) and Nayaka (2). The samples were washed with 0.3 N HCl, single and double distilled water in a sequence and air-dried. The samples were dried in paper bags at 70° C temperature till constant weight in a hot air oven and preserved for further analysis. The plant samples of grain, straw and husk were washed successively with 0.1 M HCl and then with single and double distilled water. Samples were first air dried and then at 60°-70° C temperature in a hot air oven. Dried samples were

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Table 1 : Nutrient content in rice grain samples of *Nawagam-Vatava* region .

	Rice grain			Rice straw			Rice husk		
	P	K	S	P	K	S	P	K	S
	(%)								
	Uncontaminated area								
Min.	0.13	0.17	0.18	0.11	0.58	0.11	0.11	0.56	0.15
Max.	0.19	0.25	0.32	0.32	1.29	0.23	0.21	0.89	0.24
Mean	**0.17**	**0.20**	**0.25**	**0.24**	**1.05**	**0.16**	**0.15**	**0.78**	**0.20**
	Contaminated area								
Min.	0.14	0.14	0.20	0.15	0.71	0.16	0.15	0.52	0.18
Max.	0.32	0.31	0.39	0.36	1.15	0.34	0.27	0.85	0.32
Mean	**0.22**	**0.21**	**0.30**	**0.27**	**0.94**	**0.28**	**0.22**	**0.75**	**0.28**

Table 2 : Micronutrients content in rice grain samples of *Nawagam- Vatava* region .

	Rice grain				Rice straw				Rice husk			
	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
	$\mu g\ g^{-1}$											
	Uncontaminated area											
Min.	118	17.5	20.2	1.69	102	100	16.4	7.2	71	30	14.0	2.02
Max.	257	37.8	26.3	7.63	343	314	32.5	11.9	274	143	28.0	5.55
Mean	**150**	**29.3**	**23.1**	**4.99**	**213**	**194**	**23.0**	**9.0**	**185**	**114**	**19.6**	**3.31**
	Contaminated area											
Min.	88	20.8	17.0	3.48	104	130	17.0	7.2	103	48	17.2	3.02
Max.	268	47.5	36.8	10.5	451	351	39.0	18.8	370	159	42.2	7.71
Mean	**173**	**31.1**	**29.3**	**6.99**	**279**	**231**	**25.3**	**10.5**	**224**	**120**	**24.5**	**4.90**

Table 3 : Heavy metals content in rice grain samples of *Nawagam- Vatava* region.

	Rice grain					Rice straw					Rice husk				
	Cd	Co	Cr	Ni	Pb	Cd	Co	Cr	Ni	Pb	Cd	Co	Cr	Ni	Pb
	$\mu g\ g^{-1}$														
	Uncontaminated area														
Min.	0.30	0.75	2.70	1.50	0.50	0.00	0.96	1.00	1.00	1.04	0.50	2.05	0.15	0.50	0.16
Max.	1.25	2.75	12.90	4.77	2.88	0.70	4.80	5.50	3.27	4.83	2.85	6.04	9.04	3.76	0.85
Mean	**0.89**	**2.02**	**8.62**	**2.87**	**1.44**	**0.45**	**2.72**	**3.65**	**2.24**	**2.53**	**1.32**	**3.70**	**5.21**	**1.91**	**0.53**
	Contaminated area														
Min.	0.45	1.26	8.52	2.25	1.34	0.00	1.00	3.00	1.30	0.23	1.05	1.54	6.45	0.23	0.20
Max.	1.95	4.96	18.28	7.75	6.00	1.50	7.50	9.00	6.27	4.30	2.92	6.07	18.0	2.85	1.85
Mean	**1.06**	**2.81**	**13.17**	**4.50**	**3.92**	**0.50**	**3.36**	**5.28**	**3.34**	**2.11**	**2.11**	**3.55**	**9.96**	**1.07**	**0.68**

ground in a stainless steel blade Wiley mill and digested in diacid mixture (HNO_3: $HClO_4$ - 4:1). The volume was made with double distilled water and the extract was filtered through Whatmann filter paper No.42. The acid extract was used for analysis of total P (Jackson, 1973), K (Jackson, 1973), S (Chaudhary and Cornfield 1966), trace and heavy metals (Jackson, 1973) as per standard methods.

RESULTS AND DISCUSSION

The analytical results of different parts of rice (Table 1) indicated that among the major nutrients P, K and S, the P content of rice grain and husk showed variation. The P, K and S content ranged from 0.13 to 0.19, 0.17 to 0.25 and 0.18-0.32 per cent, respectively with corresponding mean of 0.17, 0.20 and 0.25 in grain sample of rice collected form uncontaminated soil. While the mean value of P, K and S

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content were 0.22, 0.21 and 0.30 per cent in rice grain of contaminated area it ranged from 0.14 -0.32, 0.14-0.31 and 0.20-0.39 per cent, respectively. The P and S content in rice straw were noted higher in contaminated area with mean of 0.27 and 0.28 per cent, respectively as compared to uncontaminated area (Table 1), whereas K content showed revere trends. The rice husk of uncontaminated area was noted mean K content (0.78 per cent) higher than the rice husk of contaminated area (0.75 per cent). While reverse trends was observed in case of P and S content, which were higher in rice husk of contaminated area.

The analysis of different micronutrients in rice grain, straw and husk samples (Table 2) showed that the micronutrients content were higher in rice grain, straw and husk of contaminated area than in uncontaminated area. Further, it was noted that among the different micronutrients, the Cu content in rice grain and husk was 40 and 48 per cent higher than samples of uncontaminated area.

Similarly all the heavy metals also showed higher concentration in rice grain, straw and husk parts in contaminated area (Table 3). Among the heavy metals, the contamination of Pb, Cr and Ni was found quite higher in all three components of rice in contaminated area. The Cr content of grain, straw and husk of rice in contaminated area were higher by 52.8, 44.7 and 91.8 per cent over corresponding Cr content of 8.62, 3.65 and 5.21 $\mu g\ g^{-1}$ in uncontaminated area. The similarly, Ni and Pb were also higher in grain, straw and husk of rice over rice grain, straw and husk of uncontaminated area, respectively. Thus, the results clearly revealed about the presence of heavy metals in rice grain Pb, Ni and Cr showed higher contamination in affected area while in non-contaminated area, the Pb and Ni remained more concentrated in rice straw and husk, respectively. While, in case of Cr, all three components were found more contaminated in contaminated than uncontaminated area.

The analysis of chemical composition for different elements in different components of rice in contaminated as well as uncontaminated area clearly revealed about the existence of heavy metals contamination in this important crop of the *Nawagam-Vatava* region. The contamination could be ascribed to the use of contaminated tube well water for irrigation purpose. The introduction of heavy metals through the irrigation in the soil becomes a source of contamination where the farmers grow this crop. Contamination of grain with heavy metals indicated that the heavy metals are translocated to the edible portion of rice, which posing more threat to health of animal and human being if such contaminated edible portion is consumed over years. Malliwal (2005) also reported higher contamination of different crops grown along the effluent channel in vadodara region. Similar results were also reported by several workers in different plants *viz.*, Wang. (2003) in rice and Sajid (2003) in wheat.

CONCLUSION

In contaminated area of *Nawagam-Vatava* region, the heavy metal absorbance was higher by root of the rice crop as farmer using *Khari* canal water as irrigation which carrying mix industrial effluents and these heavy metal translocated in edible portion of the rice plant, which posing more threat to health of animal and human being if such contaminated edible portion is consumed over years. So, Soil-plant and ground water contamination vis-à-vis human and animal health needs to be monitored regularly in heavy metals contaminated areas as well as crop/crops species need to be identified which can eliminate or reduce the heavy metals absorption.

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Effect of Fruit Based Cropping Models on Chemical Properties of Salt Affected Soil

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The influence of 16 year old various cropping models *viz*., Aonla+Ber, Guava+Aonla, Aonla+Karonda, Guava+Ber, Guava+Karonda and Bael + Eucalyptus was studied on chemical properties *i.e.*, pHs, Electrical Conductivity (ECe), Exchangeable Sodium Percentage (ESP) and Organic Carbon (OC) of salt affected soil at 0 - 15, 15 - 30 and 30 - 45 cm depth and compared with those lying barren (control). Among the various cropping models, Guava+Ber emerged most superior than others in improving the chemical characteristics of salt affected soil through decreased pHs, ECe, ESP and increased OC. The magnitude of improvement was higher at upper depth in comparison to lower depth of soil and more in basin as compared to centre in each model.

Keywords : Salt tolerant fruit & forest species, Cropping models, Salt affected soil, Chemical properties.

INTRODUCTION

The higher amount of salt present in salt affected soils is detrimental not only to plant growth through salt imbalance but also to the physico-chemical properties of soil. Out of the total land resources available in India (3.29 million ha.), a large proportion of land is covered under salt affected soil (7.0 million ha.) and maximum area is distributed in different districts of U.P. Such category of land is ecologically and economically unproductive and need to be converted into productive land by low input means (Pandey, 2004). Reclamation of sodic soils by physical methods and chemical means is well known. Biological approach *i.e.*, growing of salt tolerant grasses, agricultural crops and fruit/forest tree species has been followed by many workers (Mishra *et al.*, 1988) with a view to improve and hence reclaim salt affected soil. Vegetation exerts a decisive influence on physical and chemical characteristics and content of organic matter through their deep root system and addition of organic matter from biomass (Nandagoudar *et al.*, 2004). This study attempts to assess the variation caused in chemical properties of salt affected soil under the influence of 16 year old various cropping models.

MATERIAL AND METHODS

The present investigation was carried out at wasteland management farm Akma of Narendra Deva University of Agriculture & Technology, Kumarganj, Faizabad, during the year 2007-08. The experiment was laid out in Randomized Block Design with 13 treatments replicating 4 times. The treatments comprised of six cropping models with two sites each *i.e.*, basin (inside canopy) and centre (outside canopy) along with control (bare site). The crop combinations were (i) Aonla+Ber, (ii) Guava+Aonla, (iii) Aonla+Karonda, (iv) Guava+Ber, (v) Guava+Karonda and (vi) Bael + Eucalyptus. Each cropping model was planted in the area of 0.5 ha and the spacings were kept 8 meter for Aonla, Guava, Ber and Bael and 4 meter for Karonda and Eucalyptus. In each model, the soil samples were taken between the centers of rows and plants and near the basins of plants. Whereas, in case of control, sampling was done from bare site (unplanted area) of the farm. The samples were randomly taken from 4 spot at 3 depths *i.e.*, 0-15, 15-30 and 30-45 cm with the help of spade, khurpi and post hole auger in the month of October-November. Soil samples were dried, grinded, sieved and used for analysis of pHs, ECe, ESP and organic carbon following standard laboratory procedures. The soil pH was tested by glass electrode pH meter in 1:2 soil water suspension. The ECe was determined by electrical conductivity meter. The organic carbon was determined as per Walky and Black's method while ESP was determined by ammonium acetate method (Richards, 1954).

RESULTS AND DISCUSSION

pHs : The perusal of data (Table-1) showed that different cropping models played vital role in reducing the soil pH and there by improving the soil. It is clear from the data that pH of the soil decreased significantly under all the cropping models over control (barren site) at different depths of soil. The pH values varied from 8.48 to 9.80 in tree models and 10.09 to 10.40 in control (barren site). Data also indicated that pH values were found to be lower at 0-15 cm depth of soil and pH increased with increasing depth of soil. The pH values were also noted to be lower in basins as compared to centres in different tree crop combinations. The pH of the soil was found to be lowest in Guava+Ber followed by Aonla+Ber. The findings

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Table 1 : Effect of different cropping models on pHs of soil.

S. No.	Treatment/ Tree cropping models		pHs at different depth (cm) 0-15	15-30	30-45	Mean value (0-45 cm)	Average value of basin and centre
1.	Control		10.09	10.33	10.40	10.27	10.27
2.	Aonla+Ber	Basin	8.63	8.81	9.40	8.94	9.07
		Centre	8.92	9.18	9.54	9.21	
3.	Guava+Aonla	Basin	8.70	8.91	9.48	9.03	9.12
		Centre	8.75	9.13	9.56	9.21	
4.	Aonla+Karonda	Basin	9.13	9.38	9.70	9.40	9.51
		Centre	9.40	9.72	9.78	9.63	
5.	Guava+Ber	Basin	8.48	8.68	8.96	8.70	8.82
		Centre	8.83	8.95	9.06	8.94	
6.	Guava+Karonda	Basin	9.08	9.25	9.43	9.25	9.34
		Centre	9.28	9.41	9.62	9.43	
7.	Bael+Eucalyptus	Basin	9.15	9.40	9.72	9.42	9.38
		Centre	9.42	9.74	9.80	9.65	
	SEm±		**0.22**	**0.23**	**0.24**		
	CD at 5%		**0.63**	**0.66**	**NS**		

Table 2 : Effect of different cropping models on ECe (dSm^{-1}) of soil.

S. No.	Treatment/ Tree cropping models		ECe (dSm^{-1}) of different depth (cm) 0-15	15-30	30-45	Mean value (0-45 cm)	Average value of basin and centre
1.	Control		14.02	13.56	12.80	13.46	13.46
2.	Aonla+Ber	Basin	6.78	7.30	7.90	7.32	7.77
		Centre	7.84	8.23	8.60	8.22	
3.	Guava+Aonla	Basin	7.28	7.54	8.01	7.61	7.91
		Centre	7.93	8.25	8.52	8.23	
4.	Aonla+Karonda	Basin	7.78	8.40	9.28	8.48	8.98
		Centre	8.88	9.68	9.93	9.49	
5.	Guava+Ber	Basin	6.38	7.08	7.84	7.10	7.66
		Centre	7.80	8.18	8.68	8.22	
6.	Guava+Karonda	Basin	7.63	8.38	9.13	8.38	8.89
		Centre	8.82	9.63	9.80	9.41	
7.	Bael+Eucalyptus	Basin	7.80	8.42	9.30	8.50	9.00
		Centre	8.90	9.70	9.95	9.51	
	SEm±		**0.19**	**0.42**	**0.30**		
	CD at 5%		**0.54**	**1.19**	**0.86**		

of Sharma and Gupta (1989), Malvia and Singh (1998) and Pandey (2004) also supported the contention that there is reduction in pH of soil by aforestation and planting of different crop plants as compared to bare site.

Electrical conductivity (dSm^{-1}) : Data pertaining to electrical conductivity (ECe) of soil as influenced by different tree cropping models indicated that ECe of soil was found to be reduced significantly in all the tree cropping models over control at different depths showing marked improvement in soil characteristics (Table-2). The ECe varied from 6.38 to 9.35 in various tree cropping models (planted area) and 12.80 to 14.02 in control (barren site). The ECe value of soil increased with increasing depth of soil irrespective of tree cropping models. Regarding canopy positions, the ECe was found to be lower in basins as compared to centres of each tree crop combination. The ECe value was found to be lowest in Guava+Ber cropping model followed by Aonla+Ber and highest in control. These findings are in line as those reported by Mishra *et al.* (1988), Singh (1985) and Pandey (2004) that the tree association considerably reduces the ECe of soil. Reduction in ECe might be due to leaching of salt, increased hydraulic conductivity

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Table 3 : Effect of different cropping models on ESP (%) of soil.

S. No.	Treatment/ Tree cropping models		ESP (%) at different depth (cm) 0-15	15-30	30-45	Mean value (0-45 cm)	Average value of basin and centre
1.	Control		71.50	75.70	80.30	75.83	75.83
2.	Aonla+Ber	Basin	32.75	36.80	45.18	38.24	41.83
		Centre	41.65	44.56	50.08	45.43	
3.	Guava+Aonla	Basin	33.65	37.66	46.58	39.29	42.63
		Centre	40.93	45.52	51.84	45.97	
4.	Aonla+Karonda	Basin	40.55	44.36	52.18	45.69	49.46
		Centre	49.75	52.94	57.00	53.23	
5.	Guava+Ber	Basin	31.65	35.86	42.00	36.50	40.39
		Centre	40.31	44.00	48.58	44.29	
6.	Guava+Karonda	Basin	39.00	43.94	49.88	44.27	47.80
		Centre	46.05	51.36	56.58	51.33	
7.	Bael+Eucalyptus	Basin	40.60	44.40	50.20	45.06	49.16
		Centre	49.80	52.98	57.04	53.27	
	SEm±		**2.30**	**1.24**	**2.68**		
	CD at 5%		**6.50**	**3.55**	**7.66**		

Table 4 : Effect of different cropping models on organic carbon (%) of soil.

S. No.	Treatment/ Tree cropping models		Organic Carbon at different depth (cm) 0-15	15-30	30-45	Mean value (0-45 cm)	Average value of basin and centre
1.	Control		0.16	0.13	0.12	0.13	0.13
2.	Aonla+Ber	Basin	0.41	0.36	0.31	0.36	0.33
		Centre	0.34	0.29	0.27	0.30	
3.	Guava+Aonla	Basin	0.39	0.33	0.29	0.33	0.30
		Centre	0.31	0.27	0.25	0.27	
4.	Aonla+Karonda	Basin	0.30	0.26	0.22	0.26	0.23
		Centre	0.23	0.20	0.19	0.20	
5.	Guava+Ber	Basin	0.45	0.38	0.33	0.38	0.35
		Centre	0.37	0.32	0.29	0.32	
6.	Guava+Karonda	Basin	0.32	0.29	0.26	0.29	0.26
		Centre	0.27	0.25	0.22	0.24	
7.	Bael+Eucalyptus	Basin	0.28	0.24	0.20	0.24	0.21
		Centre	0.21	0.18	0.17	0.18	
	SEm±		**0.02**	**0.001**	**0.01**		
	CD at 5%		**0.05**	**0.020**	**0.04**		

and decreased upward movement of salt under canopy of different cropping models.

Exchangeable sodium percentage (ESP) : It is apparent from the data (Table-3) that ESP of soil reduced considerably in planted area (cropping models) over unplanted area (barren site) at all the depths. Data also indicated that ESP of soil was found to be reduced in basin as compared to centre under all the tree cropping models. The ESP values of soil were observed to be lower at 0-15 cm depth which increased with increasing depth of soil. The average ESP of the soil was noticed lowest in Guava+Ber (40.39%) cropping model whereas, it was highest (75.83%) in control (barren site). The production of organic and in organic acids from tree biomass helps in liberating calcium for replacing harmful sodium in the exchange complex (Srivastava *et al.*, 1988). Findings of Jain and Singh (1998), Malvia and Singh (1998) and Pandey (2004) also supported the fact that ESP of the soil is reduced by plantation/afforestation in comparison to open area (bare site).

Organic carbon (%) : Data (Table-4) recorded on organic carbon status of soil revealed that organic carbon of the soil

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increased significantly in all the tree cropping models at all the depths as compared to control (barren site) indicating key point of soil improvement. It was found maximum in Guava+Ber cropping model followed by Aonla+Ber and minimum in barren site (control) at corresponding depth and canopy position. Data further indicated that organic carbon was higher in the basins of plant than centres at respective depths. It was also noticed that organic carbon was maximum in 0-15 cm layer of soil in each cropping model but it decreased with increasing depths of soil. Tomar *et al.* (1998) also stated that organic matter increased under trees as compared to bare site.

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Nutrient Optimization for Sustainable Productivity of Hybrid Maize (*Zea mays* L.) in Inseptisol

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The field experiment was undertaken to evaluate the nutrient optimization for sustainable productivity of hybrid maize (COHM 5) in Inceptisol. Nutrient sorption study was carried out to find the deficient nutrient of the experimental soil and to fix the optimum nutrient treatment. The sorption study revealed that the N, P, K, and Zn were deficient in the soil and it required as optimum nutrients of 100, 32, 24 and 2.4 mg Kg^{-1} of N, P, K and Zn for this soil. The optimum nutrient treatment (ONT) of NPK and Zn were fixed as 200:64:48 and 4.8 Kg ha^{-1} for field experiment. In each case of N, P and K there were three more levels, *viz*, a zero level, one below and another above the ONT level. The maximum plant height (159.1, 226.6 and 257.5 cm), highest drymatter production 4471 and 11126 Kg ha^{-1} were registered with the application of 250:64:48:4.8 Kg of NPK and Zn ha^{-1} at 45 and 75 DAS, respectively. Also, it influenced the yield attributes *viz*, the longest cob (19.7 cm), maximum cob girth (15.3 cm), highest number of grain rows per cob (15.4), grains row^{-1} (37.8) and hundred grains weight (28.06 g). The highest grain and stover yield of 8.01 and 14.39 t ha^{-1} were recorded for the application of 250:64:48:4.8 Kg of NPK and Zn ha^{-1} with the best net income of Rs. 38,102 ha^{-1}.

Keywords : Optimum nutrient treatment, Grain yield, Benefit : Cost ratio.

INTRODUCTION

Maize (*Zea mays* L.) the third most important cereals, next to wheat and rice in the world as well as in India. In India, maize is cultivated in 26.7 million hectares with a production of 34 million tonnes with an average yield of 1280 Kg ha^{-1}. In Tamil Nadu maize is cultivated an area of 0.18 million hectares with a production of 0.29 million tonnes with an average productivity of 1552 Kg ha^{-1} (Season and Crop Report, 2005). By 2020, the requirement of maize for various sectors will be around 100 million tonnes, of which poultry sector alone needs 31 million tonnes. Hence, it may be a very difficult task for us to increase the maize production from the present level of 34 to 100 million tonnes (Seshaiah, 2000). The concept of balanced fertilization paves the way for optimum plant nutrient supply to achieve the maximum yield potential of crop and takes care of nutrient stress of soil. A systematic approach to soil fertility evaluation as proposed by Portch and Hunter (1988) envisages the optimization of fertilizer requirement for crop based on the nutrient sorption characteristics of soil. The present study is one such attempt to evolve fertilizer optima for hybrid maize (COHM 5) in Inceptisol.

MATERIAL AND METHODS

The experiment was conducted in Ramayanpatti village of Tirunelveli district. The experimental soil belongs to Mayamankuruchi (Myk) series of *Typic* Haplusteps- Inceptisol. The chemical characteristics of the experimental soil was pH 8.07, EC - 0.26 dSm^{-1}, CEC - 31.2 c mol (p^+) Kg^{-1}, organic carbon 5.7 g Kg^{-1}, available N, P and K being 230.3, 18.4 and 292 Kg ha^{-1}, respectively. The available Zn, Fe, Cu and Mn were 2.91, 42.46, 4.26 and 20.4 mg Kg^{-1}, respectively.

Nutrient sorption study was carried out to find the deficient nutrient of the experimental soil and to fix the optimum nutrient treatment. The sorption study revealed that the N, P, K and Zn were deficient in the soil and it required as optimum nutrients of 100, 32, 24 and 2.4 mg Kg^{-1} of N, P, K and Zn for this soil. The optimum nutrient treatment (ONT) formed the central treatment ($N_2P_2K_2Zn$). The optimum nutrient treatment of NPK and Zn were fixed as 200:64:48 and 4.8 Kg ha^{-1} for field experiment. In each case of N, P and K there were three more levels, *viz*., a zero level, one below and another above the ONT level. There is one treatment which is ONT minus Zn, except this treatment all the other treatments received Zn as in ONT. Field experiment was conducted in the farmers' field with maize (COHM5) as a test crop with thirteen treatment combinations. A blanket fertilizer treatment was included for comparison. The treatments were replicated three times in a Randomized Block Design (RBD). The crop was sown on 21.06.07 and harvested on 09.10.07. The fertilizer sources used urea, single super phosphate, muriate of potash and zinc sulphate. Full dose of P, K and Zn and one third of N were applied to maize as basal at the time of sowing. The remaining

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dose of N was top dressed in 2 equal splits at 25 and 45 days after sowing. The observations on biometric and yield attributes such as plant height (45 and 75 DAS and at harvest), cob length, cob girth, grain rows cob^{-1}, number of grains row^{-1}, test weight, grain yield, dry matter production and the benefit cost: ratio were recorded and statistically analysed. The details of the treatments for this series are given below.

Treatment details for field experiment

Treat. Nos.	Treatments		Nutrients doses (Kg ha^{-1}) N	P	K	Zn
T_1	$N_0P_2K_2Zn$	:	0	64	48	4.8
T_2	$N_1P_2K_2Zn$	:	150	64	48	4.8
T_3	$N_2P_2K_2Zn$ (ONT)	:	200	64	48	4.8
T_4	$N_3P_2K_2Zn$	:	250	64	48	4.8
T_5	$N_2P_0K_2Zn$	:	200	0	48	4.8
T_6	$N_2P_1K_2Zn$	:	200	48	48	4.8
T_7	$N_2P_3K_2Zn$	:	200	80	48	4.8
T_8	$N_2P_2K_0Zn$	:	200	64	0	4.8
T_9	$N_2P_2K_1Zn$	:	200	64	36	4.8
T_{10}	$N_2P_2K_3Zn$	:	200	64	60	4.8
T_{11}	$N_2P_2K_2$-Zn	:	200	64	48	0
T_{12}	Blanket	:	135	62.5	50	5.5
T_{13}	Control	:	0	0	0	0

RESULTS AND DISCUSSION

The plant height of maize at different stages of crop growth, the grain yield and yield attributes such as cob length, cob girth, number of grain rows cob^{-1}, number of grains row^{-1} and hundred grain weight were significantly influenced by different treatments.

Growth and drymatter production : Was noticed with the application of 250:64:48:4.8 Kg of NPK and Zn ha^{-1} followed by the application of 200:64:48:4.8 Kg of NPK and Zn ha^{-1}, which gave the next maximum heights of plants *viz.*, 132.8, 211 and 224.7 cm at 45, 75 DAS and at harvest (Table. 1).

The highest drymatter production 4471 and 11126 Kg ha^{-1}, was registered in the treatment supplied with 250:64:48:4.8 Kg of NPK and Zn ha^{-1} at 45 and 75 DAS, respectively (Table. 1). Which was on par with 200:64:48:4.8 Kg of NPK and Zn ha^{-1}. The N enhanced the growth attributes such as plant height, stem diameter and leaf area index resulting in increased dry matter production. This result is in conformity with the findings of Ayub *et al.* (2002) and Hani *et al.* (2006).

Yield attributes and yield : The longest cob (19.7 cm), maximum cob girth (15.3 cm), highest number of grain rows per cob (15.4), grains row^{-1} (37.8) and hundred grains weight (28.06 g) were obtained on addition of 250:64:48:4.8 Kg of NPK and Zn ha^{-1} followed by the optimum nutrient treatment with 200:64:48:4.8 Kg of NPK and Zn ha^{-1} (19.2 cm), (14.9 cm), (14.7), (33.9) and (25.69g) (Table. 2). The blanket recommendation gave the lower values of growth and yield attributes than the ONT. This might be due to continuous supply of major nutrients at the time of its critical phases of the crop growth. Similar results were observed by Singh and Singh (2006).

Among all the treatments, significantly highest grain yield of 8.01 t ha^{-1} and stover yield of 14.39 t ha^{-1} were recorded with the application of 250:64:48:4.8 Kg of NPK and Zn ha^{-1} (Table. 2) followed by the application of 200:64:48:4.8 Kg of NPK and Zn ha^{-1} (7.3 and 12.22 t ha^{-1}). The blanket recommendation recorded the lowest yield (6.1 and 9.56 t ha^{-1}). The per cent increased in grain yields (31.7 and 185.5) over blanket and control were obtained from the treatment with 250:64:48:4.8 Kg of NPK and Zn ha^{-1} in this soil. This yield increase was mainly due to significant improvement in the yield attributes due to balanced supply of nutrients. Similar finding was also reported by Balyan *et al.* (2006).

Economics : The treatment with 250:64:48:4.8 Kg of NPK and Zn ha^{-1} had given the best net income of Rs. 38,102 ha^{-1} followed by the treatment with 200:64:48:4.8 Kg of NPK and Zn ha^{-1} with the next best net income of Rs. 32,872 ha^{-1} with the benefit cost ratio of 2.72 and 2.52, respectively. The blanket recommendation with 135:62.5:50:5.5 Kg of NPK and Zn ha^{-1} recorded the net income of Rs. 24,329 ha^{-1} with the benefit cost ratio of 2.16 (Table.2 & Fig.1).

CONCLUSION

Significant economic yield was obtained in the ONT + Zn in this soil over the blanket recommendation. Developing soil fertility evaluation at series level through ASI method for hybrid maize (COHM 5) was found to be beneficial to farmers. Hence, the application of 250:64:48:4.8 Kg of NPK and Zn ha^{-1} is recommended for realizing maximum maize yield in this soil.

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Table 1 : Effect of treatments on plant height and drymatter production of hybrid maize (COHM 5).

Treat. Nos.	Treatments	Plant height (cm)			DMP (Kg ha^{-1})	
		45DAS	75DAS	At harvest	45DAS	75DAS
T_1	$N_0P_2K_2Zn$	181.6	165.7	182.5	2482	7159
T_2	$N_1P_2K_2Zn$	73.5	175.3	195.7	3362	8865
T_3	$N_2P_2K_2Zn$ (ONT)	132.8	211.0	224.7	3557	10087
T_4	$N_3P_2K_2Zn$	159.1	226.6	257.5	4471	11126
T_5	$N_2P_0K_2Zn$	201.1	172.3	201.8	2576	7575
T_6	$N_2P_1K_2Zn$	207.5	181.3	207.6	3327	8354
T_7	$N_2P_3K_2Zn$	138.4	206.0	239.6	3422	9992
T_8	$N_2P_2K_0Zn$	100.4	163.6	184.6	2668	8401
T_9	$N_2P_2K_1Zn$	117.2	176.0	200.8	3378	8959
T_{10}	$N_2P_2K_3Zn$	122.1	183.6	228.7	3574	10019
T_{11}	$N_2P_2K_2$-Zn	98.3	161.3	198.9	2867	8133
T_{12}	Blanket	226.6	190.3	228.0	3313	8754
T_{13}	Control	71.3	146.0	173.4	2388	5521
	SEd	2.87	2.87	2.83	64.43	50.18
	CD(0.05)	5.91	5.92	5.85	132.97	103.56

Table 2 : Effect of treatments on yield parameters and grain yield of hybrid maize (COHM 5).

Treat. Nos.	Treatments	Cob length (cm)	Cob girth (cm)	Grain rows cob^{-1}	Grains row^{-1}	100 grains wt (g)	Grain yield (t ha^{-1})	Stover yield (t ha^{-1})	Net return (Rs. ha^{-1})	B:C ratio
T_1	$N_0P_2K_2Zn$	14.4	12.3	12.7	27.5	21.70	3.22	6.19	4,918	1.25
T_2	$N_1P_2K_2Zn$	15.7	13.4	14.2	30.3	23.86	6.17	10.25	25,159	2.20
T_3	$N_2P_2K_2Zn$ (ONT)	19.2	14.9	14.7	33.9	25.69	7.26	12.22	32,872	2.52
T_4	$N_3P_2K_2Zn$	19.7	15.3	15.4	37.8	28.06	8.01	14.39	38,102	2.72
T_5	$N_2P_0K_2Zn$	15.3	12.4	13.4	28.8	23.85	3.78	7.90	8,266	1.40
T_6	$N_2P_1K_2Zn$	16.3	13.6	14.3	31.2	24.59	6.31	10.11	25,143	2.16
T_7	$N_2P_3K_2Zn$	18.3	14.8	14.8	35.5	26.52	7.07	11.25	30,278	2.34
T_8	$N_2P_2K_0Zn$	16.2	13.4	13.2	29.3	24.32	3.95	7.09	8,513	1.40
T_9	$N_2P_2K_1Zn$	17.2	14.1	14.1	30.8	25.07	6.23	9.93	24,817	2.15
T_{10}	$N_2P_2K_3Zn$	18.3	14.7	14.6	35.9	26.98	7.17	11.75	31,267	2.44
T_{11}	$N_2P_2K_2$-Zn	16.0	13.1	13.2	31.2	23.34	5.86	9.25	22,721	2.08
T_{12}	Blanket	17.3	13.2	13.7	31.9	25.42	6.08	9.56	24,329	2.16
T_{13}	Control	13.3	11.8	12.2	24.8	20.61	2.80	5.51	3,957	1.23
	SEd	0.18	0.10	0.10	0.24	0.35	118.20	0.15		
	CD(0.05)	0.38	0.21	0.21	0.49	0.71	243.96	0.30		

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Studies on the Effect of Different Sources and Levels of Organic Manures on Fodder Yield and Quality in Perennial Fodder Crops

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A field experiment was conducted to evaluate the effect of different organic manures and its sources at different levels (FYM -20, 25 & 30 t ha^{-1}, vermicompost - 2.5, 5.0 & 7.5 t ha^{-1}, poultry manure - 2.5, 5.0 & 7.5 t ha^{-1} and pig manure- 2.5, 5.0 & 7.5 t ha^{-1}) on yield and quality of fodder in perennial fodder crops. The field experiment was conducted at New Area Farm, Department of Forage Crops, TNAU, Coimbatore. The experiment was laid out in randomized block design replicated twice with 14 treatments. Perennial fodder crops *viz*, cumbu napier hybrid grass CO 3, guinea grass CO 2, sorghum COFS 29, lucerne CO 1 and hedge lucerne were studied under this investigation. The results revealed that the organic manure treatments were compared with recommended dose of inorganic fertilizers with respect to each fodder crop. Among different organics, application of FYM @ 30 t ha^{-1} registered the maximum green fodder, dry fodder and crude protein yields in all perennial fodders. However the maximum green, dry fodder and crude protein yields were recorded in RDF treatment over the rest of the other treatments.

Keywords : Organic manures, Perennial fodders, Fodder yield, Fodder quality.

INTRODUCTION

Nitrogen availability from organic resources often limits to realize full yield potential of cereals under organic production system. Under restricted water availability or rainfed conditions, the differences in crop yields between organic and conventional production narrow down to between 10-15%. The nutrient rich manure helps to raise crop productivity even at lower application rates (5-10 t ha^{-1}) compared to the use of 15-20 t ha^{-1} FYM with and without chemical fertilizer (Hue, 1992). In addition, use of liquid manures prepared through fermentation of green leafy materials, cattle urine and other locally available resources are common. The differences in quality of manures used is probably the reason for wide difference reported in crop yields under organic and conventional system of crop production. There is however a need to scientifically evaluate the nutrient supply methods in organic *vs* conventional systems.

Organic farming systems rely on the management of soil organic matter to enhance the chemical, biological and physical properties of the soil. One of the basic principles of soil fertility management in organic systems is that plant nutrition depends on 'biologically- derived nutrients' instead of using readily soluble forms of nutrients; less available forms of nutrients such as those in bulky organic materials are used. This requires release of nutrients to the plant via the activity of soil microbes and soil animals. Improved soil biological activity is also known to play a key role in suppressing weeds, pests and diseases.

The supply of organic milk increased rapidly in response to market demand and financial support to convert. Although the market for organic milk and milk products continues to grow, supply now outstrips demand. Organic milk is being promoted heavily. Supermarkets are predicting strong growth for the organic sector and are promoting it strongly. The media is also very supportive. Food scares were an issue in consumer's minds, *e.g.*, BSE, *Salmonella*, *E-coli*, GMO's, misuse of antibiotics and pesticides. In addition milk buyers are canvassing producers strongly. The result of this activity is a surge in the number of farmers converting to organic production.

To produce organic milk, it is important to produce organic fodder. Organic fodder can be produced by replacing the inorganic source of fertilizers with organic manures. Sources and levels of organic manures are to be standardized without affecting the fodder yield. With this background, this study was conducted.

MATERIAL AND METHODS

A field experiment was conducted at New Area Farm of Department of Forage Crops, TNAU, Coimbatore, to evaluate the effect of different organic manures on fodder yield and quality in perennial fodder crops. Perennial fodder crops *viz.*, cumbu napier hybrid grass CO 3, guinea grass CO 2, sorghum COFS 29, lucerne CO 1 and hedge lucerne and different organic manures *viz.*, FYM, vermicompost, poultry manure and pig manure with three levels were used for investigation.

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(1) FYM - 20, 25 & 30 t ha^{-1}
(2) Vermicompost - 2.5, 5.0 & 7.5 t ha^{-1}
(3) Poultry manure - 2.5, 5.0 & 7.5 t ha^{-1}
(4) Pig manure - 2.5, 5.0 & 7.5 t ha^{-1}

The experiment was laid out in randomized block design replicated twice with 14 treatments. The organic manure treatments were compared with recommended dose of inorganic fertilizers in all the fodder crops. In organic manure treatments, full dose of organic manures was applied basally as per the treatment schedule. In inorganic fertilizer treatments, the recommended dose of NPK fertilizers (Cumbu napier hybrid- 150:50:40 Kg ha^{-1}; Guinea grass - 150:40:40 Kg ha^{-1}; Sorghum - 90:40:40 Kg ha^{-1}; Lucerne - 80:120:20 Kg ha^{-1} and Hedge lucerne - 80:120:20 Kg ha^{-1}) were applied to all fodder crops. 50% of nitrogen and full dose of phosphorus and potassium were applied as basal and remaining 50% of nitrogen was applied as top dressing for the first cutting in all fodder crops. For the subsequent cuttings, 50% of the nitrogen was applied as basal after each harvest. The plot size for each treatment is 40sq.m. The experiment was conducted for three years.

Crops were harvested at 50% flowering stage in lucerne, hedge lucerne and sorghum COFS 29 where as in cumbu napier grass and guinea grass, harvesting was done at 50-55 days after planting. Seven cuttings were harvested in cumbu napier hybrid, guinea grass, lucerne and hedge lucerne in one year whereas in sorghum COFS 29, five cuttings were harvested in one year. After each harvest, green fodder yield was recorded in all crops. Dry matter yield was computed by assessing the moisture percentage for all crops. Plant samples were collected at harvest and analysed for crude protein content. From dry matter yield and crude protein content, crude protein yield was computed in all fodder crops. The organic manures *viz.*, FYM, vermicompost, poultry manure and pig manures taken for the study were analysed for nutrient contents and furnished as follows.

Nutrients	FYM	Vermic-ompost	Poultry Manure	Pig Manure
Nitrogen (%)	0.50	1.50	1.20	0.55
Phosphorus (%)	0.20	0.50	0.40	0.50
Potassium (%)	0.50	0.80	0.40	0.40

RESULTS AND DISCUSSION

Green fodder yield : Among different organic manures, application of FYM @ 30 t ha^{-1} registered the maximum and superior green fodder yield of 218.1 t ha^{-1} yr^{-1}. This is followed by the treatments *viz.*, vermicompost @ 7.5 t ha^{-1}, poultry manure @ 7.5 t ha^{-1} and FYM @ 25 t ha^{-1} and gave on par green fodder yield (210.1 t ha^{-1} yr^{-1}, 194.9 t ha^{-1} yr^{-1} and 200.6 t ha^{-1} yr^{-1} respectively). In guinea grass, FYM @ 30 t ha^{-1} recorded the maximum green fodder yield (145.6 t ha^{-1} yr^{-1}) followed by FYM @ 25 t ha^{-1} (130.6 t ha^{-1} yr^{-1}), vermicompost @7.5 t ha^{-1} (133.3 t ha^{-1} yr^{-1}) and poultry manure @ 7.5 t ha^{-1} (118.3 tha^{-1} yr^{-1}). In sorghum COFS 29, application of FYM @ 30 t ha^{-1} registered superior green fodder yield (161.6 t ha^{-1} yr^{-1}) and on par yields were recorded by FYM @ 25 t ha^{-1}, vermicompost @ 5.0, 7.5 t ha^{-1} and poultry manure @ 7.5 t ha^{-1} (145.9 t ha^{-1} yr^{-1}, 123.3 t ha^{-1} yr^{-1}, 148.5 t ha^{-1} yr^{-1} and 131.7 t ha^{-1} yr^{-1} respectively). In legume fodder crops *viz.*, lucerne CO 1 and hedge lucerne, application of FYM @ 30 t ha^{-1} recorded the maximum green fodder yield of 71.2 t ha^{-1} yr^{-1} and 104.4 t ha^{-1} yr^{-1} (Table 1). The maximum green fodder yield was recorded in inorganic fertilizer treatment (T 14) over the rest of the treatments in all the perennial fodders.

Gangwar and Niranjan (1991) reported similar increase in dry matter production and green fodder yield of sorghum by the application of FYM alone @ 15 t ha^{-1}. Adequate biomass production, better nutrient uptake and improvement in yield parameters might have resulted in higher tuber yield in cassava consequent to application of composted poultry manure either alone or in combination with FYM (Pillai *et al.*, 1987)

Dry fodder yield : The results on dry fodder yield are presented in Table 2. Application of farm yard manure @ 30 t ha^{-1} was the superior treatment in recording the highest dry fodder yield in cumbu napier hybrid CO 3 (52.4 t ha^{-1}), guinea grass CO 2 (33.5 t ha^{-1}), sorghum COFS 29 (35.6 t ha^{-1}), lucerne CO1 (19.9 t ha^{-1}) and hedge lucerne (27.2 t ha^{-1}). Application of FYM @ 25 t ha^{-1}, vermicompost @ 5.0, 7.5 t ha^{-1} and poultry manure @ 7.5 t ha^{-1} gave on par yields of 48.2 t ha^{-1}, 42.6 t ha^{-1}, 50.4 t ha^{-1} and 46.8 t ha^{-1} respectively in cumbu napier hybrid grass. Similar trend of results were observed in other fodder crops also. The quick release and supply of nutrients through farm yard manure @ 30 t ha^{-1} might have increased the protoplasmic constituents and elongation. This in turn resulted in increased fodder yield. Similar results were reported by Dudhat *et al.*, (1996).

Fodder quality : Application of FYM @ 30 t ha^{-1} recorded the maximum crude protein yield in all perennial fodder crops (Cumbu Napier Hybrid CO 3 - 5.50 t ha^{-1}: Guinea grass-2.38 t ha^{-1}: Sorghum COFS 29- 3.01 t ha^{-1}: Lucerne CO 1: 4.01 t ha^{-1}: Hedge Lucerne - 5.40 t ha^{-1}) (Table 3) which was on par with vermicompost application @ 7.5 t ha^{-1}. Similar kind of results were obtained by Pholsen *et al.* (2001) by applying FYM in fodder maize. No significance difference was observed in crude fibre content in all perennial fodder crops due to the application of different sources of organic manures (Table 4).

It was concluded that to produce organic fodder and to achieve maximum green fodder yield, FYM @ 30 t ha^{-1} is to be applied. Improved fodder quality was observed in all perennial fodders with respect to crude protein yield due to the application of FYM @ 30 t ha^{-1} and vermicompost @ 7.5 t ha^{-1}.

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Table 1 : Effect of different organic manures on green fodder yield of different perennial fodders (t ha^{-1} yr^{-1}).

Treatments	Cumbu Napier Hybrid CO 3	Guinea grass CO 2	Sorghum COFS 29	Lucerne CO 1	Hedge lucerne
T1-Control	88.1	55.1	74.1	35.7	60.4
T2-FYM 20 t ha^{-1}	187.7	104.1	120.9	50.1	80.3
T3-FYM 25 t ha^{-1}	200.6	130.6	145.9	61.7	92.6
T4-FYM 30 t ha^{-1}	218.1	145.6	161.6	71.2	104.4
T5-Vermicompost 2.5 t ha^{-1}	173.1	101.8	118.1	45.1	75.2
T6-Vermicompost 5 t ha^{-1}	185.7	108.3	123.3	50.2	80.2
T7-Vermicompost 7.5 t ha^{-1}	210.1	133.3	148.5	66.8	99.8
T8-Poultry Manure 2.5 t ha^{-1}	157.2	92.60	107.6	45.9	68.0
T9-Poultry Manure 5 t ha^{-1}	169.8	109.4	120.4	50.5	80.9
T10-Poultry Manure 7.5 t ha^{-1}	194.9	118.3	131.7	63.1	94.2
T11-Pig Manure 2.5 t ha^{-1}	110.1	85.00	104.0	45.8	63.8
T12-Pig Manure 5 t ha^{-1}	119.9	101.8	113.2	50.8	78.5
T13-Pig Manure 7.5 t ha^{-1}	128.6	110.7	120.5	56.6	81.9
T14-Recommended NPK*	310.2	185.0	171.0	85.7	118.7
SEd	27.4	15.6	18.5	9.51	10.9
CD(0.05)	59.2	33.8	39.9	20.5	23.5

*Cumbu napier hybrid -150:50:40 Kg ha^{-1}; Guinea grass -150:40:40 Kg ha^{-1}; Sorghum -90:40:40 Kg ha^{-1}; Lucerne -80:120:20 Kg ha^{-1} and Hedge lucerne - 80:120:20 Kg ha^{-1}

Table 2 : Effect of different organic manures on dry fodder yield of different perennial fodders (t ha^{-1} yr^{-1}).

Treatments	Cumbu Napier Hybrid CO 3	Guinea grass CO 2	Sorghum COFS 29	Lucerne CO 1	Hedge lucerne
T1-Control	21.2	12.7	16.3	10.0	15.7
T2-FYM 20 t ha^{-1}	40.1	25.3	26.5	14.2	21.0
T3-FYM 25 t ha^{-1}	48.2	30.0	32.1	17.3	24.1
T4-FYM 30 t ha^{-1}	52.4	33.5	35.6	19.9	27.2
T5-Vermicompost 2.5 t ha^{-1}	40.6	23.4	25.9	14.1	19.6
T6-Vermicompost 5 t ha^{-1}	42.6	25.2	26.3	14.2	20.7
T7-Vermicompost 7.5 t ha^{-1}	50.4	30.7	32.7	18.7	25.9
T8-Poultry Manure 2.5 t ha^{-1}	37.7	21.3	23.7	14.3	17.7
T9-Poultry Manure 5 t ha^{-1}	40.8	25.2	26.7	15.4	21.1
T10-Poultry Manure 7.5 t ha^{-1}	46.8	27.2	29.3	17.7	24.5
T11-Pig Manure 2.5 t ha^{-1}	26.4	19.6	22.9	12.8	16.6
T12-Pig Manure 5 t ha^{-1}	28.8	23.4	24.9	15.4	20.4
T13-Pig Manure 7.5 t ha^{-1}	30.9	25.3	27.8	16.8	22.4
T14-Recommended NPK*	74.5	42.6	37.6	22.6	30.9
SEd	5.24	3.59	4.06	2.66	2.82
CD(0.05)	11.3	7.76	8.77	5.75	6.10

*Cumbu napier hybrid -150:50:40 Kg ha^{-1}; Guinea grass -150:40:40 Kg ha^{-1}; Sorghum -90:40:40 Kg ha^{-1}; Lucerne -80:120:20 Kg ha^{-1} and Hedge lucerne -80:120:20 Kg ha^{-1}

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Table 3 : Effect of different organic manures on crude protein yield (t ha^{-1}).

Treatments	Cumbu Napier Hybrid CO 3	Guinea grass CO 2	Sorghum COFS 29	Lucerne CO 1	Hedge lucerne
T1-Control	2.22	0.90	1.38	2.01	3.13
T2-FYM 20 t ha^{-1}	4.23	1.82	2.21	2.82	4.18
T3-FYM 25 t ha^{-1}	5.06	2.14	2.72	3.48	4.80
T4-FYM 30 t ha^{-1}	5.50	2.38	3.01	4.01	5.40
T5-Vermicompost 2.5 t ha^{-1}	4.30	1.67	2.20	2.83	3.89
T6-Vermicompost 5 t ha^{-1}	4.48	1.82	2.39	3.39	4.12
T7-Vermicompost 7.5 t ha^{-1}	5.29	2.18	2.77	3.76	5.17
T8-Poultry Manure 2.5 t ha^{-1}	3.96	1.52	2.01	2.83	3.52
T9-Poultry Manure 5 t ha^{-1}	4.28	1.79	2.27	3.29	4.14
T10-Poultry Manure 7.5 t ha^{-1}	4.91	1.94	2.46	3.56	4.88
T11-Pig Manure 2.5 t ha^{-1}	2.94	1.39	1.94	2.58	3.30
T12-Pig Manure 5 t ha^{-1}	3.02	1.67	2.11	3.09	4.26
T13-Pig Manure 7.5 t ha^{-1}	3.24	1.81	2.36	3.38	4.66
T14-Recommended NPK	7.82	3.02	3.19	4.55	6.14
SEd	0.55	0.26	0.35	0.53	0.56
CD(0.05)	1.19	0.55	0.75	1.16	1.21

*Cumbu napier hybrid -150:50:40 Kg ha^{-1}; Guinea grass -150:40:40 Kg ha^{-1}; Sorghum -90:40:40 Kg ha^{-1}; Lucerne -80:120:20 Kg ha^{-1} and Hedge lucerne -80:120:20 Kg ha^{-1}

Table 4 : Effect of different organic manures on crude fibre content (%).

Treatments	Cumbu Napier Hybrid CO 3	Guinea grass CO 2	Sorghum COFS 29	Lucerne CO 1	Hedge lucerne
T1-Control	30.0	35.9	24.3	21.2	17.9
T2-FYM 20 t ha^{-1}	30.1	36.0	24.6	21.3	18.1
T3-FYM 25 t ha^{-1}	30.1	36.1	24.7	21.4	18.5
T4-FYM 30 t ha^{-1}	30.1	36.2	24.8	22.7	18.7
T5-Vermicompost 2.5 t ha^{-1}	29.9	36.2	24.1	21.1	18.5
T6-Vermicompost 5 t ha^{-1}	29.9	36.2	24.3	21.8	18.5
T7-Vermicompost 7.5 t ha^{-1}	29.9	36.4	24.3	21.9	18.5
T8-Poultry Manure 2.5 t ha^{-1}	30.1	36.4	23.8	21.0	18.2
T9-Poultry Manure 5 t ha^{-1}	30.1	36.5	23.9	21.3	18.3
T10-Poultry Manure 7.5 t ha^{-1}	30.1	36.6	24.0	21.5	18.3
T11-Pig Manure 2.5 t ha^{-1}	30.1	36.3	23.7	21.2	18.2
T12-Pig Manure 5 t ha^{-1}	30.1	36.3	23.6	21.5	18.4
T13-Pig Manure 7.5 t ha^{-1}	30.1	36.4	23.7	21.7	18.6
T14-Recommended NPK*	30.6	37.3	25.8	22.9	18.9
SEd	-	-	-	-	-
CD(0.05)	NS	NS	NS	NS	NS

*Cumbu napier hybrid -150:50:40 Kg ha^{-1}; Guinea grass -150:40:40 Kg ha^{-1}; Sorghum -90:40:40 Kg ha^{-1}; Lucerne -80:120:20 Kg ha^{-1} and Hedge lucerne -80:120:20 Kg ha^{-1}

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Effect of Position of the Cuttings and Indole Butyric Acid on the Root Induction in Culm Cuttings of Five Commercially Important *Dendrocalmus* species

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In the absence of seeds, the propagation through rooting of culm cuttings has been found to be successful in many bamboo species. A study was conducted on the influence of position of the cuttings and different concentrations of IBA on the root induction in culm cuttings of five bamboo species belonging to *Dendrocalmus* genus. Three noded cuttings were prepared from the basal, middle and top portion of the culms and treated with 0, 100 and 200 ppm of IBA. The interaction effects of species, position of the cuttings and IBA treatment was significant at 5 per cent level. With regards to position of cuttings, rooting response was higher from the basal cuttings in *D. asper, D. longispathus* and *D. stocksii*, middle and top cuttings in *D. gigantueus* and top cuttings in *D. membranaceus*. The application of IBA enhanced the rooting response in all bamboo species and in *D. asper, D. giganteus* and *D. longispathus*, the best rooting response was obtained with 100 ppm IBA treatment. Whereas, in *D. membranaceous* and *D. stocksii* treatment with 200 ppm IBA resulted in better rooting response. The selection of culm cuttings based on the position of the cuttings and application of proper concentration of growth regulating substances can be resorted to obtain the efficient rooting of the culm cuttings in these bamboo species.

Keywords : *Dendrocalamus* species, Propagation, Culm cuttings, Rooting, IBA treatment, Bamboo.

INTRODUCTION

There is an ever increasing demand for bamboo due to multifarious and versatile uses. Propagation through seeds is considered to be the cheapest method in bamboo planting stock production. However, non-availability of seeds due to long flowering cycle and poor viability makes the conventional vegetative propagation methods such as offset planting, layering and rooting of culm cuttings etc. inevitable in planting stock production of various bamboo species. The propagation through rooting of culm cuttings has been found to be successful in many bamboo species (Banik 1980; Singh *et al.* 2006) and the rooting ability varies with species. The success of root induction in the culm cuttings depends on various factors such as season of taking the cuttings, growth regulating substances and their concentration, position of the cuttings etc (Banik, 1980; Surendran and Seethalakshmi, 1985). Investigations by various scientists had proved summer as the ideal season for rooting and the 2 to 3 years old culm cuttings as the ideal material for better rooting response (Seethalakshmi *et al.* 1990; Agnihotri and Ansari, 2003; Singh *et al.* 2004 & 2006). Hence, we conducted a study on the influence of position of node and concentrations of IBA on root induction in culm cuttings of five important bamboo species *viz.*, *D. asper, D. giganteus* Munro, *D. longispathus* Kurz, *D. membranaceous* Munro and *D stocksii* as. (Munro) M. Kumar, Ramesh and Unnikrishnan.

MATERIAL AND METHODS

The experiment was carried out at Kerala Forest Research Institute Field Research Centre Campus, Veluppadam, Kerala. The study area is located at 10°26' 31" North latitude and 76°21' 36.9" East longitude and the area is gently undulating with an elevation of 40 MSL. The soil was loamy sand and slightly acidic.

During December 2006, 2-3 year old culms were extracted from the 20 year old bamboo clumps of *D. asper, D. giganteus, D. longispathus, D. membranaceous* and *D. stocksii* that are grown in Veluppadam campus and being extracted for planting stock production at regular intervals. The culms were measured and divided into bottom, middle and top portions and three noded cuttings were prepared from each portion of the culm. The cuttings were treated with 0, 100, 200 ppm IBA (Indole Butyric Acid, Merk) by cavity method (KFRI, 1990). Five, three-nodded cuttings constituted one treatment

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and it was replicated thrice. The treated cuttings were laid on standard propagation beds and planted horizontally in trenches. The propagation beds were provided with 50 per cent shade using green shade nets and were uniformly watered twice daily for the initial two months and then at an interval of two days except on rainy days. Water logging was strictly avoided. The sprouted cuttings were uprooted after six months of planting and observations were made on number and height of sprouts, rooting percentage, number of roots and root length.

Statistical analysis : Bartlett's test of sphericity on the matrix of correlation coefficients among the variables (sprouting and rooting attributes) was significant at 1 % level ($\chi^2 = 332.693$) which indicated the suitability of factor analysis to the present data set. Factor analysis was conducted using SPSS (Norusis, 1988). To find the best species, position and treatment combination, a cluster analysis was carried out using average linkage method and squared Euclidian distance as distance measure (Hair *et al.*, 2005) taking the treatment combinations as entities and sprouting and rooting parameters as characters to find the best treatment combination. The individual effects of position of cuttings and IBA in rooting attributes were compared using 'lsd' (least significant difference).

RESULTS AND DISCUSSION

Number of days required for root initiation : The culm cuttings initiated sprouting after the first week of planting in *D. giganteus*. Whereas, *D. asper, D. longispathus, D. membranaceous* and *D. stocksii* sprouted in second week (Table 1). Rooting of the cuttings initiated after the completion of sprouting. Cuttings rooted as early as third week in *D. longispathus*. Whereas, *D. asper* and *D. giganteus* initiated rooting in fourth week. Rooting initiated slowly in *D. membranaceous* and *D. stocksii* (fifth and sixth week respectively). The rooting was completed after a period of eight to ten weeks in all the species. Banik (2008) had reported 60-70 days forrooting in *D. giganteus* and 55-70 days in *D. stocksii*.

Effect of species, position of the cuttings and IBA treatment on sprouting and rooting attributes of bamboo species : Dendrogram of the Cluster analysis identified eight homogenous clusters at 20 % variance level; the treatments 34-43 the first group, 9-36 the second group, 25 the third, 39, 45, 38 and 42 the fourth, 17-2 the fifth, 3 the sixth, 5 seventh and 6 the eight group (Figure 1). The treatment combinations falling with in a cluster are homogenous in nature. When the sprouting and rooting parameters of the treatment combinations were compared, the clusters 6, 7 and 8 were superior to other clusters and the least values were recorded by the cluster 1 (Table 2). Basal cuttings of *D. asper* treated with IBA 200 ppm, treatment number 3 produced the highest rooting percentage (70.67) with 53.67 roots of length 44.67 cm. There were 3.93 sprouts per node with a height of 2.34 m. The next highest rooting was observed in the middle cuttings treated with IBA 100 ppm (64.33 %) and which was followed by the middle cuttings treated with 200 ppm IBA (60.57 %); treatments 5 and 6 respectively. Middle cuttings treated with 100 ppm IBA produced 40 roots with an average length of 93.00 cm and 3 sprouts per node that are 1.93 m high. Those treated with IBA 200 ppm produced 36.17 roots of length 31.5 cm and 2.33 sprouts that are 0.67 m high. It has been demonstrated that the thick walled bamboo species respond better than thin walled bamboo species (Surendran *et al.*, 1983; Seethalakshmi *et al.*, 1990) in adventitious root induction. Our study is consistent with this finding, all five thick walled bamboos had shown a good rooting response.

Effect of position of the cuttings on the rooting attributes of the bamboos species : In *D. asper*, the average rooting was the highest from the basal cuttings (41.22%) and the basal cuttings produced significantly higher number of roots (35.67) per node. The rooting percentage of cuttings from different parts of the culm was at par in *D. giganteus*. But higher number (34.22) and length (41.50 cm) of roots was obtained in middle cuttings. In *D. longispathus* basal cuttings possessed higher rooting (39.11 %), number (32.42) and length of the roots (47.22 cm). There was no significant variation in rooting parameters of the *D. membranaceous* due to position of the cutting and middle cuttings even completely failed to root. In *D. stocksii*, the number and length of the roots were at par in cuttings from different culm positions. As the rooting of top cuttings was found to be lower in *D. asper* and *D. stocksii*, it can be excluded during large scale planting stock production to reduce the nursery cost.

Effect of IBA treatment on the rooting attributes of bamboo species : The rooting attributes of bamboo species as influenced by different concentrations of IBA are given in the Table 4. The cuttings treated with IBA produced higher rooting response compared to control in all bamboo species. In *D. asper, D. giganteus* and *D. longispathus*, the best rooting response was obtained with 100 ppm IBA treatment. Whereas, in *D. membranaceous* and *D. stocksii* treatment with 200 ppm IBA resulted in better rooting response. The positive response of growth regulating substances such as NAA, IBA, and chemicals such as boric acid, coumarin etc on root induction in culm cuttings also has been reported in earlier works (Surendran and Seethalakshmi, 1985; Surendran *et al.* 1986; Agnihotri and Ansari, 2000; Singh *et al.*, 2006).

Statistical analysis : Factor analysis identified a single factor which explained 59.37 percentage of the total variance. ANOVA of the factor scores revealed that the interaction effects, species x position of the node (p=0.01), species x IBA (p=0.01) and species x position of the node x IBA (p=0.05) were significant.

Table 1 : The time taken for sprouting and rooting of culm cutting in different bamboo species.

Species	Sprouting begins (dap)	Sprouting ends (dap)	Rooting starts (dap)
D. asper	9	14	22
D. giganteus	6	25	28
D. longispathus	12	16	19
D. membranaceous	13	18	40
D. stocksii	14	24	31

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Table 2 : Interaction effect of species, position of node and IBA treatment on sprouting and rooting attributes.

Treatment No.	Species	Position	GRS (ppm)	No. of sprouts	Sprout height (m)	Rooting %	Root number	Root length
1.	*D. asper*	Base	Control	0.00	0.00	0.00	0.00	0.00
2.			IBA100	2.63	2.14	53.00	53.33	69.83
3.			IBA200	3.93	2.34	70.67	53.67	44.67
4.		Middle	Control	0.00	0.00	0.00	0.00	0.00
5.			IBA100	3.00	1.93	64.33	40.00	93.00
6.			IBA200	2.33	0.67	60.57	36.17	31.50
7.		Top	Control	0.00	0.00	0.00	0.00	0.00
8.			IBA100	2.67	1.97	49.00	39.33	63.67
9.			IBA200	3.00	1.29	13.00	14.67	28.67
10.	*D. giganteus*	Base	Control	0.00	0.00	0.00	0.00	0.00
11.			IBA100	3.00	1.89	37.33	50.00	38.33
12.			IBA200	2.67	2.20	35.00	40.40	42.67
13.		Middle	Control	1.33	1.27	13.00	27.00	30.00
14.			IBA100	2.17	1.49	37.33	38.50	38.00
15.			IBA200	2.00	2.31	33.00	37.17	55.67
16.		Top	Control	0.00	0.00	0.00	0.00	0.00
17.			IBA100	2.33	1.37	42.00	25.10	45.67
18.			IBA200	3.00	2.50	30.67	51.37	58.00
19.	*D. longispathus*	Base	Control	3.33	0.97	22.00	28.67	27.67
20.			IBA100	5.33	2.15	53.33	34.67	71.33
21.			IBA200	2.67	1.69	42.00	33.93	42.67
22.		Middle	Control	0	0	0	0	0
23.			IBA100	2.67	1.67	39.67	39.33	39.57
24.			IBA200	1.67	1.58	21.67	16.00	30.33
25.		Top	Control	0.67	40.00	11.00	6.00	7.67
26.			IBA100	2.73	1.73	48.67	37.63	58.13
27.			IBA200	2.23	1.28	37.33	25.00	36.67
28.	*D. membranaceous*	Base	Control	0.00	0.00	0.00	0.00	0.00
29.			IBA100	1.33	0.77	8.67	8.00	18.67
30.			IBA200	2.00	1.17	11.00	8.33	17.00
31.		Middle	Control	0.00	0.00	0.00	0.00	0.00
32.			IBA100	0.00	0.00	0.00	0.00	0.00
33.			IBA200	0.00	0.00	0.00	0.00	0.00
34.		Top	Control	0.00	0.00	0.00	0.00	0.00
35.			IBA100	0.00	0.00	0.00	0.00	0.00
36.			IBA200	4.23	0.79	26.67	12.53	18.00
37.	*D.stocksii*	Base	Control	3.67	0.51	40.00	8.03	22.00
38.			IBA100	4.65	0.74	67.00	9.50	30.50
39.			IBA200	4.77	0.97	55.67	14.00	37.83
40.		Middle	Control	4.33	0.30	17.67	6.33	9.17
41.			IBA100	3.00	0.50	40.00	6.47	15.07
42.			IBA200	3.83	0.91	46.50	9.07	40.83
43.		Top	Control	0.33	0.06	2.00	3.33	12.00
44.			IBA100	0.67	0.22	15.33	4.93	8.90
45.			IBA200	7.83	0.70	59.77	11.03	35.67

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Table 3 : The rooting attributes of different bamboo species as influenced by the position of the node.

Species	Position	Rooting percentage	Root number	Root length (cm)
D. asper	Base	41.22[a]	35.67[a]	38.17[ab]
	Middle	31.63[b]	25.39[b]	41.50[a]
	Top	20.67[c]	18.00[c]	30.78[b]
D. giganteus	Base	24.11[a]	30.13[ab]	27.00[b]
	Middle	27.78[a]	34.22[a]	41.22[a]
	Top	27.22[a]	25.49[b]	34.56[ab]
D. longispathus	Base	39.11[a]	32.42[a]	47.22[a]
	Middle	20.44[b]	18.44[b]	23.30[c]
	Top	32.33[a]	22.88[b]	34.16[b]
D. membranaceous	Base	6.56[a]	5.44[a]	11.89[a]
	Middle	0.00[a]	0.00[a]	0.00[a]
	Top	8.89[a]	4.18[a]	6.00[a]
D. stocksii	Base	54.22[a]	10.51[a]	30.11[a]
	Middle	34.72[b]	7.29[a]	22.69[a]
	Top	25.7[b]	6.433[a]	21.86[a]

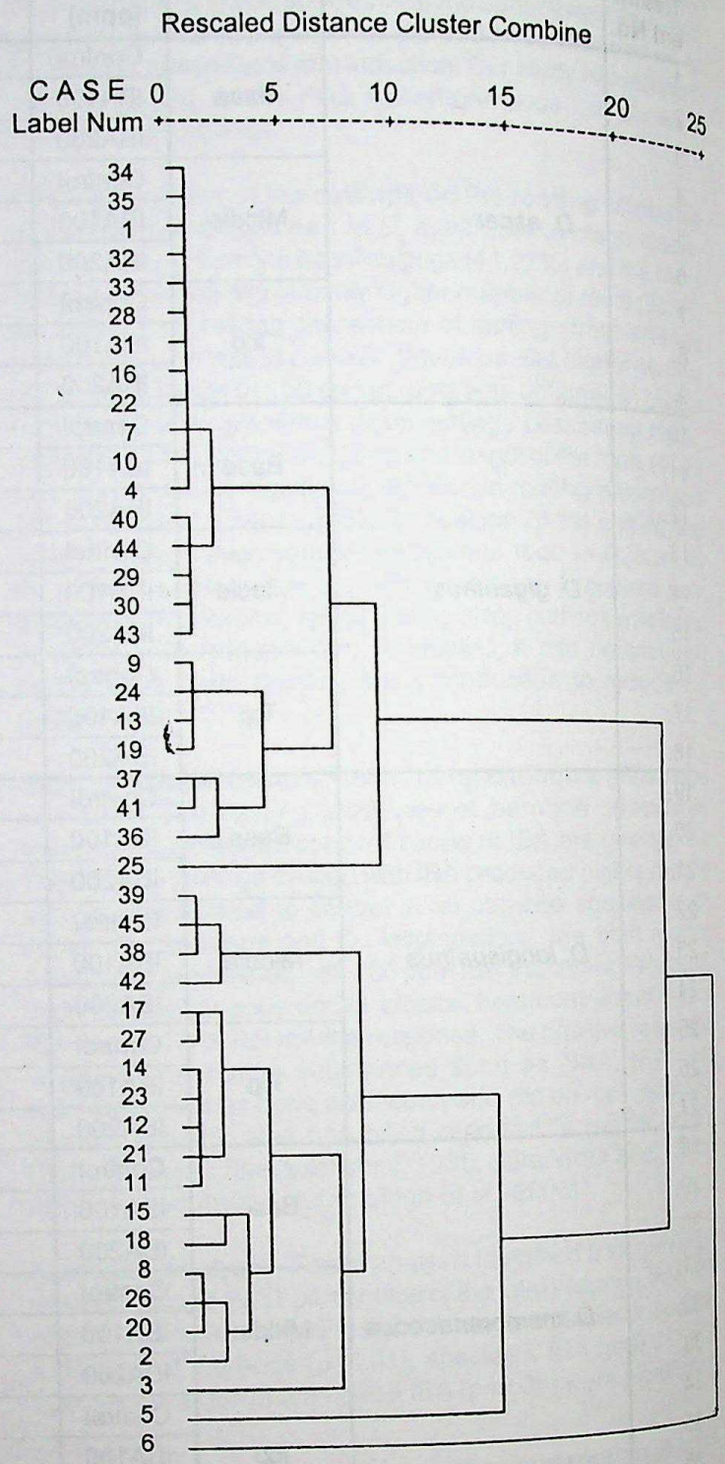
Note: means within a species and in a column having same letter as superscript are homogeneous.

Table 4 : The rooting attributes of different bamboo species as influenced by different concentrations of IBA.

Species	Treatment (ppm)	Rooting percentage	Root number	Root length (cm)
D. asper	Control	0.00[c]	0.00[c]	0.00[c]
	IBA 100	55.44[a]	44.22[a]	75.50[a]
	IBA 200	38.08[b]	34.83[b]	34.94[b]
D. giganteus	Control	4.33[b]	9.00[b]	10.00[c]
	IBA 100	38.89[a]	37.87[a]	40.67[b]
	IBA 200	35.89[a]	42.98[a]	52.11[a]
D. longispathus	Control	11.00[c]	11.56[c]	11.78[c]
	IBA 100	47.22[a]	37.21[a]	56.34[a]
	IBA 200	33.67[b]	24.98[b]	36.56[b]
D. membranaceous	Control	0.00[b]	0.00[b]	0.00[b]
	IBA 100	2.89[b]	2.67[ab]	6.22[ab]
	IBA 200	12.56[a]	6.96[a]	11.67[a]
D. stocksii	Control	19.89[c]	5.90[a]	14.39[b]
	IBA 100	40.78[b]	6.97[a]	18.16[b]
	IBA 200	53.98[a]	11.37[a]	38.11[a]

Note: means within a species and in a column having same letter as superscript are homogeneous.

Figure 1 : The generated dendrogram showing different clusters.



In summary, the rooting of culm cuttings in bamboos varied with species. The position of the cuttings influenced the rooting performance and it varied with the species. In *D. asper* and *D. stocksii*, during the large scale planting stock production the cuttings from top portion of the culms can be excluded to reduce the cost of production. As the influence of IBA concentrations also was found to be significant, application of proper concentration of IBA also can enhance rooting response in the culm cuttings as well as reduce the cost of seedling production.

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"Front Line Demonstration - An Effective Tool for Increasing the Productivity of Groundnut and Soybean in Jalgaon District of Maharashtra"

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Groundnut and soybean are the important oilseed crops widely consumed in India, which plays a major role in supplementing the income of small and marginal farmers of Jalgaon district (M.S.). The front line demonstration (FLD) were conducted by the Krishi Vigyan Kendra, Pal ,Dist - Jalgaon (M.S.) with improved package of practices of groundnut and soybean cultivation for three years (2006-07 to 2008-09) and achieved the expected yields as compared to district productivity. The yield of groundnut and soybean crops can be increased by demonstrating their cultivation technologies at the farmer's field under the supervision of scientists working in the operational area. During the period under study it was observed that yield of demonstration was significantly higher (groundnut 1805 Kg/ha, soybean 2,490 Kg/ha) than the farmers practices (local check). In all the years front line demonstration shows a significant increase in yield of demonstrations over farmer practices.

Keywords : Groundnut, Soybean, Front line demonstration, Yield.

INTRODUCTION

Groundnut and soybean are the important oilseed and cash crop, which has a vital role in Indian agriculture, industry and export trade. These crops are commercially and nutritionally very important source of oil and proteins. Groundnut kernels are rich in vitamin A, B & E. Because of high nutritive value and its rich oil percentage, soybean cultivation is increasing day by day.

India ranks first in the World in groundnut area (7.30 million ha.) and its rank in second in the production (8.30 million Ton) but productivity is almost 1/3rd to USA (Anonymous, 2000). Similarly the planted area under soybean in India is above 8.88 million ha, which produces above 9.99 million tones of soybean with an average productivity 1,124 Kg. ha^{-1}.

Now a days oilseed are more beneficial to the farmers in terms of money as compared to cereals. Groundnut and soybean crops are grown as a cash crop in the conutry. Keeping in views of above facts Front Line Demonstration is introduction by the Indian Council of Agricultural Research, New Delhi, with the inception of technology mission of pulse and oilseed crops during mid eightles. The basic objectives of FLD are to speedy spread of the newly introduced high yielding variety of groundnut and soybean and acquaint extension functionaries and local farmers with front line varieties and management technologies.

MATERIAL AND METHODS

Front line demonstration on groundnut and soybean was conducted by Krishi Vigyan Kendra, Pal, Dist - Jalgaon (M.S.) during 2006-07, 2007-08 and 2008-09 in *Kharif* season, in six villages of two tahsils, Raver and Yawal. The total 109 farmers were associated under this programme. The demonstration of improved technology was taken in 0.40 ha. area of each farmers. Total 45 ha. area was covered in 3 years the demonstration of recommended improved practices of groundnut and soybean. In the demonstration, one control plot was also kept where farmer practices was carried out. The result was compared with the full package of practices. The primary data was collected from the selected FLD farmers with the help of interview schedule and converted and presented in the term of percentage and the qualitative data were converted into quantitative form and expressed in term of present increased yield was calculated by using following formula.

$$\% \text{ increased yield} = \frac{\text{Demonstration yield} - \text{Farmers yield}}{\text{Farmers yield}} \times 100$$

RESULTS AND DISCUSSION

Perusal of data (Table 1 & 2) revealed that under demonstration plots, groundnut and soybean yield was found

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Table 1 : Difference between demonstration package and farmers practice under FLD on groundnut and soybean.

Particulars	Groundnut		Soybean	
	Demonstration	Farmer practices	Demonstration	Farmer practices
Variety	JL-286 JL-501	Local	DS-228	Local
Seed rate	120 Kg/ha	120 to 130 Kg/ha	75 Kg/ha.	100 to 110 Kg/ha
Seed treatment	Thirum - 5 gm/Kg seed	No seed treatment	Carbendizm-2.5 gm/Kg seed	No seed treatment
Situation	Rainfed	Rainfed	Rainfed	Rainfed
Fertilizer dose	25:50:00 (NPK) Kg.ha^{-1}	20:40:00 (NPK) Kg.ha^{-1}	50:75:00 (NPK) Kg.ha^{-1}	25:50:00 (NPK) Kg.ha^{-1}
Plant protection	Need based insecticides and fungicides spray	No spray of insecticides and fungicides spray	Need based insecticides and fungicides spray	Injudicious use of DP chemicals

Table 2 : Increasing the productivity of groundnut through Front line demonstration.

Year	Under FLD Programme		Average yield (Kg.ha^{-1})		% yield increase over farmers practices	Extension gap (Kg.ha^{-1})	C.B. Ratio
	Total farmers	Total area (ha.)	FLD	Farmers practices			
2006-07	13	5.00	1,468	1,039	41.28	429	2.57
2007-08	20	10.00	1,805	1,150	56.96	655	2.82
2008-09	25	10.00	1,596	1,117	42.88	479	2.45
Total/average	**58**	**25.00**	**1,623**	**1,102**	**47,27**	**521**	**2.61**

Table 3 : Increasing the productivity of Soybean through Front line demonstration.

Year	Under FLD Programme		Average yield (Kg.ha^{-1})		% yield increase over farmers practices	Extension gap (Kg.ha^{-1})	C.B. Ratio
	Total farmers	Total area (ha.)	FLD	Farmers practices			
2006-07	13	5.00	1,980	1,315	50.57	665	2.82
2007-08	13	5.00	2,490	1,810	37.57	680	2.67
2008-09	25	10.00	2,210	1,635	35.17	575	2.16
Total/average	**51**	**20.00**	**2,227**	**1,587**	**40.32**	**640**	**2.55**

Table 4 : Area and productivity of different oilseed crop in Jalgaon district (Maharashtra) during *Kharif* 2006-07.

Crop	Area (ha.)	Productivity (Kg.ha^{-1}.)
Maharashtra		
Groundnut	4,50,000	889
Soybean	25,20,000	1,147
Jalgaon		
Groundnut	6,3000	1,112
Soybean	14,600	1,620

(Source: Department of Agriculture, Govt. of Maharashtra)

substantially higher than that under farmers practice during all the year. Under different location, the groundnut yield in demonstration plots ranged between 1468 to 1805 Kg.ha^{-1} over observation period, which was 41.28 to 56.96 per cent higher over farmer practices (local check). On overall basis, 47.27 per cent increase in yield was recorded. Similarly soybean yield in demonstration plots ranged between 1980 to 2490 Kg.ha^{-1} over observation period, which was 35.17 to 50.57 per cent higher over farmer practices (local check). On overall basis 40.32 per cent increase in yield was recorded. These results are in conformity with the findings to Tomar *et al.* (2003), in front line demonstration on soybean and of Tiwari and Sexsena (2001) and Tiwari *et al.* (2003) in other crops.

Yield improvement of groundnut and soybean mainly due to knowledge and adoption of appropriate production technology. Use high yield varieties JL-286, JL-501 of groundnut and DS-228 of soybean crops, appropriate sowing time, seed treatment, method of fertilizer application and plant protection practices adopted under the front line demonstration. The above findings are in similarly with the finding of Singh (2002). Similarly Kirar *et al.* (2005) also emphasized the important of FLD in soybean production.

The economic analysis given in the Table 2 and 3 indicates that the cost benefit (C.B) ratio in groundnut crop

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ranged from 2.45 to 2.82 with mean value of 2.61 in the form of increased yield. Similarly, in soybean crop the cost benefit (C.B.) ratio ranged from 2.16 to 2.82 with a mean value of 2.55 in the form of increased yield. These results are in conformity with the findings of Billore *et al.* (2004), Katare *et al.* (2009).

CONCLUSION

By conducting front line demonstrations of proven technologies, yield potential of groundnut and soybean can be increased to a great extent. This is concluded that FLD programme was effective for increasing productivity as well as in changing attitude, skill and knowledge of improved practices of high yielding varieties of groundnut and soybean including adoption. The demonstration acts also as source of such valuable information of other farmers.

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Fresh Seed Dormancy in Promising Genotypes of Groundnut (*Arachis hypogaea* L.)

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The field experiment was conducted in summer season on clay soil at Main Oilseeds research station, Junagadh Agricultural University, Junagadh to study the fresh seed dormancy in promising genotypes of groundnut (*Arachis hypogaea* L.). The genotypes developed from crosses between the Spanish-type varieties were studied and compared with check varieties for fresh seed dormancy over three years. The intensity of dormancy ranged from 16% for the genotype INS-II-2003-3 to 100 % for genotype ISK-II-2003-1. These latter genotype exhibited dormancy duration longer than 15 days after harvest while, the genotype ISK-II-2003-1 showed a period of dormancy of less than one week days after harvest. On the basis of the dormancy's scale used, the rate of the degree of seed dormancy for the genotypes ranged below level 6 for the genotypes AG-2238, JB-1077, JB-1078, NS-I-2001-6, INS-II-2003-3, INS-II-2003-4, ICGV-92121 which was the least dormant genotypes to above level 7 for the genotypes DRT-2004-6, INS-I-2003-2, ISK-II-2003-1, INS-I-2003-4, ISK-II-2003-19, and DRT-2004-17 which were the most dormant genotypes. These genotypes used as donor parents for incorporation of fresh seed dormancy.

Keywords : Groundnut, Genotypes, Intensity, Fresh seed dormancy.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a major cash crop in the semi-arid tropics, where it is mostly grown under rainfed conditions (Ntare *et al.*, 2001). India ranks first in area (8.00 Million hectares) and second in production (7.50 million tonnes) in the world (Anon., 2003). In Gujarat, the Saurashtra region is one of the most drought prone areas and it contributes 85 and 81 per cent of area and production, respectively and thus it is considered to be the "groundnut bowl" of the country. Many times, drought in sub- tropics regions has induced a shortening of the rainy season and an erratic rainfall. These climatic changes led to a prevalence of cultivars in environments out of their recommended area. The main focus of the groundnut improvement program carried out in to create early groundnut varieties that could survive with drought that may occur at the end of the rainy season period (Annerose, 1990 and Khalfaoui,1991). However, such early varieties belonging to Spanish-type usually lack fresh seed dormancy. Then rains that may fall at the harvest period could cause *in situ* germination. This experience is a major problem in Saurashtra region of Gujarat state. Gautreau (1984) found that the yield losses due to sprouts of Spanish varieties are considerable and range from 10 to 20% of the pod yield. Moreover, the sprouting is known to increase the susceptibility of peanut to *Aspergillus* infection, which can result in aflatoxin contamination (Martin *et al.*, 1999). Then, sprouting may also result low seed quality. Therefore, testing or breeding for fresh seed dormancy in peanut is important in environments, where rains may occur after peanut maturity. Spanish varieties released with fresh seed dormancy still remain limited because of the little number the studied involving.

The objectives in this study were (i) to find out the genotypes having fresh seed dormancy (ii) to estimate the intensity of dormancy of promising groundnut genotypes (iii) to estimate the duration of their fresh seed dormancy and rank them using the scale of Landfort *et al.* (1965).

MATERIAL AND METHODS

Since last many years' 280 entries of bunch groundnut genotypes from breeding trials have been screened for presence of fresh seed dormancy (FSD). Entries having less than 30 per cent germination were selected for evaluating for FSD. Promising genotypes were maintained in a nursery and repeatedly tested for dormancy during summer and *Kharif* seasons. Out of these, fifteen promising groundnut genotypes (E_1-AG-2238, E_2-JB-1077, E_3-JB-1078, E_4-NS-I-2001-6, E_5-INS-I-2003-2, E_6-INS-I-2003-4, E_7-INS-I-2003-6, E_8-INS-II-2003-3, E_9-INS-II-2003-4, E_{10}-ISK-II-2003-1, E_{11}-ISK-II-2003-19, E_{12}-DRT-2004-6, E_{13}-DRT-2004-15, E_{14}-DRT-2004-17, and E_{15}-ICGV-92121) were identified with consistent good performance. These fifteen groundnut genotypes were compared to release varieties used as check varieties. The released varieties C_{16}-GG-2, C_{17}- TG-26, C_{18}- GG-7 are commonly cultivated in the

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"Gujarat State" and are short maturing varieties (95 days) but without fresh seed dormancy and most susceptible to *in situ* germination, they need to be tested under replicated trial for their duration, intensity of their fresh seed dormancy. Hence, this experiment was planned.

The experiment was cultivated at Main Oilseeds research station, Junagadh Agricultural University, Junagadh. The experimental design was randomized complete block design with three replications. Each replication consisted of 15 bunch groundnut genotypes+3 checks (GG-2, TG-26 and GG-7). The soil of the experimental plot was clayey in texture and slightly alkaline in reaction. The soil has an organic carbon content of 0.54 per cent and was medium in available nitrogen and potash and sulphur, low in phosphorus. The moisture content of the experimental plot at field capacity and permanent wilting point were 28.4 and 12.8 per cent, respectively, while the bulk density was 1.42 g cm^{-3}. The different genotypes of groundnut were sown in the 1st week of July in all the years at a spacing of 45 cm x 10 cm. The entire dose of fertilizers 12.5 $KgNHa^{-1}$ and 25 $KgP_2O_5ha^{-1}$ was applied basal as per recommendations. Normal cultural practices were followed during the growing season. At harvest (during the fall season), a sample of mature pods was randomly collected from each entry for the fresh seed dormancy test. The pods were shelled and enough caution was taken to prevent any damage of the seed components (testa, cotyledons and embryo). Prior to the planting, the seeds were spread with a fungicide. The maturity of the pods was assessed by the blackening of the internal inner parenchyma of the pod (Miller and Burns, 1971). Since apical seeds are known to be less dormant than basal seeds (Ketring and Morgan, 1970). Therefore, all the tests in this study were performed using apical seeds.

A test was performed in Petri-dishes washed with sodium hypocrite (46°) in laboratory without using anyincubator. Hundred seeds randomly selected were sown in a Petri-dish using filter paper moistened with distilled water and replicated four times for each entry. During the test, the mean day/ night temperature in the laboratory was 35/27°C. The filter paper was maintained moist by adding distilled water every day. Seed was considered to germinate as soon as the radical appeared.

Seed germination counts were taken at seven and fifteen days after harvesting from each treatment. The percentage of germinated seeds for entry at a given date was calculated by the following formula: Germination (%) = Number of germinated seeds * 100/ Total number of seeds. Seed dormancy is characterized by its intensity and duration. These parameters were estimated using the method suggested by Kumar *et al.* (1991). According to these latter authors, intensity of dormancy is defined as the percentage of seeds that not germinated seven (7) days after the sowing. The duration of dormancy was measured by the number of days taken to attain 30% of germinated seeds. The scale devised by Landfort *et al.* (1965) was used to rate the degree of dormancy. It was performed on data collected from on investigation.

RESULTS AND DISCUSSION

Germination Per cent: The percentages of germinated seeds are summarized in Table 1. The lowest percentages of germinated seeds were observed with genotypes E_6 -INS-I-2003-4, E_7 -INS-I-2003-6 and E_{10} -ISK-II-2003-1 in years 2005, 2007 and 2008. For a given genotypes, percentages of germination have varied slightly from one year to another. In fact, beyond environmental conditions such as temperature and moisture, others non-genetic factors could influence the seed germination in groundnut (Toole *et al.*, 1964). The genotypes E_5-INS-I-2003-2, E_6-INS-I-2003-4, E_7-INS-I-2003-6, E_{10}-ISK-II-2003-1, E_{11}-ISK-II-2003-19, E_{12} -DRT-2004-6, E_{13} -DRT-2004-15, E_{14} -DRT-2004-17 have shown less than 30 percentages of germinated seeds at 7 and 15 days after harvest.

Intensity of dormancy : Intensity of dormancy average of three years was presented in Table 2. The intensity of dormancy ranged from 100% to 16% at 7 DAH and 96% to 5% at 15 DAH was observed. The finding is in agreement with the results of Faye *et al.* (2009) and Kumar *et al.* (1991) with others genotypes. Such large variations in the intensity of dormancy could be related to genetic differences between the genotypes tested. The genotypes E_6-INS-I-2003-4, E_7-INS-I-2003-6 and E_{10} -ISK-II-2003-1 showed the highest values of intensity of dormancy in both periods. While the non-dormant varieties and genotypes E_4-NS-I-2001-6, E_8-INS-II-2003-3 and E_{15}-ICGV-92121 exhibited the lowest intensity of dormancy.

Duration of Dormancy : The genotypes showed different dormancy duration (Table 3). It ranged from more than 15 DAH to less than 7 DAH. Genotypes E_5-INS-I-2003-2, E_6-INS-I-2003-4, E_7-INS-I-2003-6, E_{10}-ISK-II-2003-1, E_{11}-ISK-II-2003-19 and E_{14} -DRT-2004-17 were persistently the more dormant genotypes. Their duration of dormancy was more than 15 DAH. In contrast, the check varieties (GG-2, GG7 and TG-26) and others genotypes have shown dormancy duration less than 15 DAH. These results were consistent with the findings of many authors Pandya and Patel (1986) and Wadia *et al.* (1987). In fact, they argued that there's genetic variability for seed dormancy among Spanish-type peanut varieties. It is found that intensity and duration of dormancy seemed to be correlated as it was reported previously by Kumar *et al.* (1991) in some of the varieties research used in his study. Actually, the more the intensity of dormancy was the longer was dormancy duration (Table 2, 3) meaning that these genotypes showed strong and long duration seed dormancy. As the intensity of dormancy, the duration of dormancy for a given entry varied more or less over seasons due to non-genetic factors. Many authors Toole *et al.* (1964); Ketring and Morgan (1970) and Kumar *et al.* (1991) reported that seed dormancy is influenced by environmental conditions and moreover the mother-plant environment may have influence on the dormancy of the seeds. When the scale of Landfort *et al.* (1965) was used to rate the degree of seed dormancy of the entries, they laid on different levels (Table 4). While the susceptible check varieties (GG-2, GG-7 and TG-26) and non-dormant genotypes was on level 2 to 4, the most dormant genotype (E_{10} -ISK-II-

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Table 1 : Germination percentage promising bunch groundnut genotypes.

Sr. No.	Name of entry	Germination per cent							
		2005		2007		2008		Average	
		At 7 DAH*	At 15 DAH	At 7 DAH	At 15 DAH	At 7 DAH	At 15 DAH	At 7 DAH	At 15 DAH
E_1	AG-2238	36	48	36	60	42	56	38	55
E_2	JB-1077	60	67	52	65	50	60	54	64
E_3	JB-1078	72	78	48	80	66	96	62	85
E_4	INS-I-2001-6	92	92	68	84	76	92	79	89
E_5	INS-I-2003-2	12	12	10	18	12	16	11	15
E_6	INS-I-2003-4	4	4	4	8	4	8	4	7
E_7	INS-I-2003-6	0	4	4	8	0	8	1	7
E_8	INS-II-2003-3	92	96	80	96	80	92	84	95
E_9	INS-II-2003-4	4	32	0	32	4	40	3	35
E_{10}	ISK-II-2003-1	0	0	0	4	0	8	0	4
E_{11}	ISK-II-2003-19	12	18	8	20	8	20	9	19
E_{12}	DRT- 2004-6	4	12	16	32	16	32	12	25
E_{13}	DRT- 2004-15	32	44	18	18	20	24	23	29
E_{14}	DRT- 2004-17	20	26	10	10	12	24	14	20
E_{15}	ICGV-92121	92	94	40	92	48	96	60	94
C_{16}	GG-2	24	24	56	94	56	100	45	73
C_{17}	TG-26	68	68	84	96	76	96	76	87
C_{18}	GG-7	72	78	72	96	72	96	72	90

* Days after harvest

Table 2 : Intensity of dormancy of groundnut genotypes at 7 and 15 days after harvest (Average data of 3 years).

Sr. No.	Genotypes	Intensity of dormancy (%)	
		7 DAH	15 DAH
E_1	AG-2238	62	45
E_2	JB-1077	46	36
E_3	JB-1078	38	15
E_4	INS-I-2001-6	21	11
E_5	INS-I-2003-2	89	85
E_6	INS-I-2003-4	96	93
E_7	INS-I-2003-6	99	93
E_8	INS-II-2003-3	16	5
E_9	INS-II-2003-4	97	65
E_{10}	ISK-II-2003-1	100	96
E_{11}	ISK-II-2003-19	91	81
E_{12}	DRT- 2004-6	88	75
E_{13}	DRT- 2004-15	78	71
E_{14}	DRT- 2004-17	86	80
E_{15}	ICGV-92121	40	6
C_{16}	GG-2	55	27
C_{17}	TG-26	24	13
C_{18}	GG-7	28	10

2003-1) were in the highest level of dormancy (level 8). Therefore, we observed from the rating that the tested genotypes were dispatched into two groups depending on the level of dormancy (Table 4). Definitely, we noted that genotypes E_1, E_2, E_3, E_4, E_8, E_{13}, E_{15} and the check varieties (GG-2, GG-7 and TG-26) formed the group in which entries were non-dormant. However, genotype E_{13} was better than three check varieties. The remaining genotypes (E_5, E_6, E_7, E_9, E_{10}, E_{11}, E_{12}, and E_{14}) set another group in which the entries were actually dormant.

CONCLUSION

Genotypes shown different intensity and duration of fresh seed dormancy. Particularly, it was noted that DRT-2004-6, INS-I-2003-2, ISK-II-2003-1, INS-I-2003-4, ISK-II-2003-19 and DRT-2004-17 possessed Fresh Seed Dormancy of more than 15 days; these most promising genotypes will be prepared for official registration. Our results suggested that the dormant genotypes could be used as donor parent in breeding programs and it could use an *in vitro* test to reliably evaluate the level of dormancy on groundnut genotypes.

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Table 3 : Duration of dormancy of genotypes tested over three years.

Sr. No.	Genotypes	Different degree of dormancy		
		2005	2006	2008
E_1	AG-2238	<7	<7	<7
E_2	JB-1077	<7	<7	<7
E_3	JB-1078	<7	<7	<7
E_4	INS-I-2001-6	<7	<7	<7
E_5	INS-I-2003-2	>15	>15	>15
E_6	INS-I-2003-4	>15	>15	>15
E_7	INS-I-2003-6	>15	>15	>15
E_8	INS-II-2003-3	<7	<7	<7
E_9	INS-II-2003-4	15	15	15
E_{10}	ISK-II-2003-1	>15	>15	>15
E_{11}	ISK-II-2003-19	>15	>15	>15
E_{12}	DRT- 2004-6	>15	15	>15
E_{13}	DRT- 2004-15	7	>15	15
E_{14}	DRT- 2004-17	>15	>15	>15
E_{15}	ICGV-92121	<7	<7	<7
C_{16}	GG-2	>15	<7	<7
C_{17}	TG-26	<7	<7	<7
C_{18}	GG-7	<7	<7	<7

Table 4 : Genotypes classified using the scale of Landfort *et al.* (1965).

Percentage of not germinated seeds (%)	Dormancy scale	Genotypes
0	0	-
0-10	1	-
11-20	2	E_8
21-40	3	E_3, E_4, E_{15}, C_{17}, C_{18}
41-60	4	E_2, C_{16}
61-70	5	E_1
71-80	6	E_{13}
81-99	7	E_5, E_6, E_7, E_9, E_{11}, E_{12}, E_{14}
100	8	E_{10}

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Studies on Packaging Technology for Long Term Storage of *Jasminum grandiflorum* L. Flowers

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A study was conducted to standardize a packaging technology for long term storage of loose flowers of *Jasminum grandiflorum*. Laboratory experiments were laid out in FCRD with two factors (chemical treatments and packaging). The treatments comprised of 12 chemical treatments, packing in 4 types of polypropylene bag without vent (PP 20 µ, 40 µ, 60 µ and 80 µ) and packaging in CFB (corrugated fibre board) boxes with 4% vent and storage under cold room conditions. It was found that the packaging technology (50 ppm salicylic acid+ pp 60 µ packing and CFB packaging) stored under cold room conditions, extended the shelf life of flowers up to 156.32 hours. The visual parameters such as freshness index, flower opening index, colour retention index, fragrance index and the physiological parameters such as moisture content, physiological loss in weight (PLW), relative water content and membrane integrity were also studied in relation to their influence in extending the shelf life of flowers.

INTRODUCTION

J. grandiflorum called as 'Pitchi/Jathimalli' in Tamil and 'Jasmine' in English is a large shrub with intensely fragrant single flowers, pink in bud stage and snow white when fully open. Pin type, Thrum type, Bangalore, Coimbatore, Thimmapuram, CO-1 and CO-2 are some of the clones of this species (Abdul Khader and Kumar, 1995). No proper estimate of area and production of Jasmine in India is available. Among the Indian states, Tamil Nadu is the leading producer of Jasmine in India with an annual production of 76,671 tonnes from 9893 hectares of *J. sambac* and 7641 tonnes from 849 hectares *J. grandiflorum* (Tamil Nadu State Department of Horticulture Report, 2006).

The flowers are used for making garlands and bouquets, adoring hair of women, religious offering etc. They are also used for the production of essential oils in the form of 'concrete' and 'absolute' which are used in cosmetic and perfumery industries. Besides the above uses, in the recent years, export of the jasmine flowers have also picked up because of their increasing demand from the Indian population settled in the Middle East countries and United States of America. These floweres are very delicate and show signs of wilting with an abrupt loss of fragrance within 24-36 hours after harvesting. Whenever there is delay in sending the flowers due to flight cancellations, the exporters face heavy losses, because the flowers cannot be preserved beyond a day. Since the flowers retain their freshness only for a day in ordinary packaging, any packaging technology that would extend the storage life of flowers under such conditions for even few hours would definitely benefit the exporters. Hence the present study was undertaken at the Department of Floriculture and Landscaping of the HC & RI of TNAU, to standardize an ideal package (chemical dip, storage temperature and packing material) for long term storage of jasmine flowers.

MATERIAL AND METHODS

Laboratory trials were conducted through several experiments. Fresh unopened flower buds of *J. grandiflorum* formed the experimental material. Experiments were laid out in FCRD with two factors (Chemical treatment and polypropylene bag packing). Details of chemical treatments are as follows, T_1 - Sucrose 2%, T_2 - Sucrose 4%, T_3 - Boric acid 2%, T_4 - Boric acid 4%, T_5 - Salicylic acid 25 ppm, T_6 - Salicylic acid 50 ppm, T_7 - Ascorbic acid 50 ppm, T_8 - Ascorbic acid 100 ppm, T_9 - NAA 50 ppm, T_{10} - NAA 100 ppm, T_{11}- Distilled water, T_{12} - No soaking (control). Four types of polypropylene bags (PP 20 µ, 40 µ, 60 µ and 80 µ) were used for packing flowers. 10 g of fresh flower buds of uniform size were treated with the chemicals, surface dried, packed in polypropylene bags and heat sealed. These bags in turn were packaged in CFB boxes lined with newspapers, sealed with packing tapes and stored under cold room conditions. The temperature and relative humidity of the cold room were 7-8 °C and 80-85 % respectively, as per the recommendation of Bose and Raghava (1975).

Visual observations (freshness index, flower opening index, colour retention index, fragrance index and Shelf life (hours) were recorded as per the sensory evaluation scoring of Madhu (1999). Physiological parameters such as moisture content, physiological loss in weight (PLW), relative water content and membrane integrity were also evaluated. Observations were recorded on the 3rd and 6th day of storage.

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Shelf life of the flowers was recorded in hours when 50 per cent of the flowers showed signs of wilting. The standard procedure of Sukhatme and Amble (1985) was adopted in the statistical scrutiny of the data.

RESULTS AND DISCUSSION

Effect of Polypropylene packing (P)

Visual parameters : Both after 3 days and 6 days of storage, packing of flowers in PP 60 μ (P_3) resulted in maximum freshness index of 79.80 per cent 47.26 per cent (Table 1) and maximum colour retention index of 86.29 and 72.68 per cent respectively when compared to all other treatments. PP 80 μ (P_4) recorded the least flower opening index of 11.35 and 16.60 per cent (Table 1) both after 3 and 6 days of storage.

Physiological parameters : All other physiological parameters (Table 3 and 4) were found significant with PP 60 μ (P_3) which recorded the least PLW (1.01 and 2.64 per cent) and maximum values for moisture content (45.14 and 32.10 per cent), RWC (73.13 and 51.99 per cent) and membrane integrity (71.31 and 83.52 per cent) after 3 and 6 days of storage respectively. P_3 registered the maximum shelf life of 128.30 hours (Table 5).

Packing of flowers in polypropylene (PP) bags proved beneficial in improving the shelf life of flowers by creating a passive modified atmosphere for the flowers. Hall *et al.,* (1975) has demonstrated the suitability of polymer films for packaging of products which prevents gas and moisture transmission and slows down the respiration. Significant extension of the post harvest life of jasmine and tuberose flowers with polypropylene packing has been reported earlier in rose (Bredmose, 1980), chrysanthemum (Yamashita *et al.*, 1999) and gladiolus (Singh and Kumar, 2008). Maintenance of maximum freshness in flowers in the present study might be due to higher levels of moisture content, RWC and membrane integrity maintained in the PP 60 μ bags which in turn have led to least PLW.

Effect of chemical treatment (T)

Visual parameters : Among the chemical treatments, both after 3 and 6 days after storage, salicylic acid 50 ppm (T_6) recorded the maximum freshness index of 93.75 and 64.11 per cent (Table 1) and maximum colour retention index of 94.44 and 77.22 per cent (Table 2) respectively. Least flower opening index was registered by Sucrose 2% (T_1) with 17.19 and 25.75 per cent (Table 2) after 3 and 6 days of storage respectively.

Physiological parameters : The treatment salicylic acid 50 ppm (T_6) also recorded significant values for the physiological parameters (Table 3 and 4) such as least PLW (0.31 and 1.79 per cent), maximum values for moisture content, RWC and membrane integrity (52.65, 85.30 and 61.55 per cent) after 3 days and (38.35, 62.13 and 75.83 per cent) after 6 days of storage respectively. A shelf life of 150.43 hours (Table 5) was also recorded by T_6.

Salicylic acid treatment of *J. grandiflorum* flowers exhibited significance in prolonging the shelf life of flowers under cold room conditions by maintaining higher levels of moisture content, RWC and least levels of physiological loss in weight and solute leakage. In agreement with the present finding, extended vase life of rose (Li *et al.*, 1999), carnation (Petridou *et al.* 1999) and China aster (Ramesh, 1999) has been reported with salicylic acid treatment. It has been reported that salicylic acid improved the longevity of flowers by blocking the conversion of 1-ACC to ethylene (Leslie and Romani, 1986). Salicylic acid possesses chelating properties (Hew, 1987) and is an anti-transpirant and a biocide which improves the flower quality and longevity by blocking the conversion of 1-ACC to ethylene (Leslie and Romani, 1986). Also a decreased rate of respiration after treatment with salicylic acid has been observed in roses (Li *et al.*, 1999). All these findings lend support to the present finding pertaining to salicylic acid.

Effect of P x T interaction

Visual parameters : In the interaction effect, after 3 days of storage, pp 60 μ (P_3) and PP 80 μ (P_4) recorded 100 per cent freshness and colour retention index (Table 1 and 2) on interaction with sucrose 2% (T_1), sucrose 4% (T_2), salicylic acid 25 ppm (T_5) and salicylic acid 50 ppm (T_6). After 6 days of storage, the interaction of salicylic acid 50 ppm + PP 60 μ (P_3T_6) proved significant and registered the maximum freshness index of 80.00 per cent (Table 1). Salicylic acid 25 ppm + PP 60 μ (P_3T_5) and Salicylic acid 50 ppm + PP 60 μ (P_3T_6) were found to be on par with each other (Table 2) with regard to colour retention index (88.88%). Distilled water + PP 80 μ (P_4T_{11}) recorded the minimum flower opening index (2.50 and 7.75 per cent) both after 3 and 6 days of storage.

Physiological parameters : For all the physiological parameters (Table 3 and 4), both after 3 and 6 days after storage, salicylic acid 50 ppm + PP 60 μ (P_3T_6) recorded significant values, least PLW (0.31 and 1.00 per cent), maximum moisture content (56.19 and 44.80 per cent), maximum RWC (91.03 and 73.99 per cent), maximum membrane integrity (56.95 and 67.90 per cent) and maximum shelf life of 156.32 hours (Table 5). All the chemical treatments (T_1 to T_{12}) and packages (P_1 to P_4) and their interaction registered a fragrance score of '3' (strong) up to 3 days of packing and a score of '2' (mild) after 6 days of packing.

In the present investigation, the variation noticed among the packing x chemical treatment (PxT) interaction was more significant than their individual effects. The combination of chemical treatments and MAP of flowers might have recorded a significant freshness index of the flowers by maintaining their moisture content, relative water content and membrane integrity at favourable levels, thereby delaying the wilting of flowers.

Flower opening : Least flower has been recorded by PP 80 μ which might due to its low O_2 permeability rate when compared to other PP packs. Also the fragrance level of flowers remained strong upto 3 days of packing which might be due to the maintenance of freshness of flowers indicating an active respiration state of flowers. Thereafter until 6 days, there might have been build up of CO_2 with negligible levels of O_2 causing

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the flowers to undergo anaerobic respiration, finally leading to evolution of an undesirable fragrance.

Moisture content and Relative water content : Under cold room conditions, most of the packages recorded moisture content and relative water content on par with each other after 3 days of storage. The relative water content of the flowers manifests the water status of the flower petals. It is obvious that when moisture content is more and weight loss is less, relative water content stays at higher levels. In the present study, moisture levels of 40 to 50 per cent have resulted in 'nil' PLW which in turn have registered higher relative water content of 70 to 90 per cent. Thus, those packages with significant moisture content and RWC proved to be on par with each other in respect of their potentials in maintaining the final quality and post harvest life of the flowers. After 3 days, a gradual declining trend in moisture content has been noticed with a simultaneous decline in RWC and an increase of solute leakage. These factors might have caused the difference in the freshness index as recorded by the packages.

Physiological loss in weight : Physiological loss in weight (PLW), moisture content, relative water content (RWC) and membrane integrity of flowers are inter-related to each other. Increased PLW leads to decline in fresh weight of flowers, which is expressed visually as senescing symptoms such as wilting of flowers as reported in carnation (Nichols, 1966) and in *Rosa damascena* (Sharma 1981). A similar declining trend in fresh weight with an abrupt weight loss during senescence has been reported earlier in gerbera (Burzo *et al.*, 1992), tulip (Jones *et al.*, 1995) and China rose - *Rosa hybrida* cv. Samantha (Xue and Lin, 1999). Evidence has been reported in gladiolus flower senescence wherein a decrease in RWC (relative water content) of tepals caused the dehydration of tissues and in turn wilting (Zahed Hossain *et al.*, 2006).

Membrane integrity : The loss in membrane integrity as a symptom of flower senescence could be attributed to the fact that petal wilting is a result of loss of turgor pressure of cells. The changes in membrane permeability and as associated water loss as symptoms of petal wilting have been established in *Ipomoea* (Hanson and Kende, 1976) and in *Tradescantia* (Woltering *et al.*, 1993). Solute leakage reflecting membrane deterioration reported in carnation flowers (Paulin *et al.*, 1986), *Rosa hybrida* 'Samantha' (Xue and Lin, 1999) also supports this result.

SUMMARY AND CONCLUSION

The package involving a combination of 50 ppm salicylic acid + PP 60 µ packing + CFB packaging + cold room storage recorded maximum freshness, colour retention and an extended shelf life to 156.32 hours. This package also proved beneficial in maintaining the physiological parameters of flowers to a fevourable extent. Hence this packaging technology can be used for long term storage of *J. grandiflorum* flowers.

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Table 1 : Effect of chemical treatments and different packaging material on freshness index (%) and flower opening index (%) *J. grandiflorum*.

Packing / Treatment	Freshness index (%)										Flower opening index (%)									
	3 days after packing					6 days after packing					3 days after packing					6 days after packing				
	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean
T_1 - Sucrose 2%	89.28 (71.66)	81.42 (64.69)	100 (88.34)	100 (88.34)	92.68 (78.26)	48.42 (44.09)	57.14 (49.11)	74.28 (59.62)	28.57 (32.29)	52.10 (46.28)	18.75 (25.64)	26.25 (30.80)	12.50 (20.69)	11.25 (19.58)	17.19 (24.18)	27.50 (31.61)	33.75 (35.50)	25.25 (30.15)	16.50 (23.95)	25.75 (30.30)
T_2 - Sucrose 4%	66.66 (54.77)	72.85 (58.68)	100 (88.34)	100 (88.34)	84.88 (72.53)	28.57 (32.29)	57.14 (49.11)	26.42 (30.92)	28.57 (32.29)	35.18 (36.15)	25.00 (29.98)	28.75 (32.41)	7.50 (15.88)	15.00 (22.77)	19.06 (25.26)	28.75 (32.41)	43.75 (41.40)	18.75 (25.64)	20.25 (26.73)	27.88 (31.55)
T_3 - Boric acid 2%	47.85 (43.76)	57.14 (49.11)	57.14 (49.11)	45.71 (42.53)	51.96 (46.13)	28.57 (32.29)	28.57 (32.29)	22.14 (28.05)	28.57 (32.29)	26.96 (31.23)	16.25 (23.76)	23.75 (29.15)	20.00 (26.55)	6.25 (14.47)	19.56 (23.48)	30.00 (33.20)	43.75 (41.40)	43.75 (41.40)	11.50 (19.81)	32.25 (33.44)
T_4 - Boric acid 4%	46.42 (42.94)	57.14 (49.11)	57.14 (49.11)	45.71 (42.53)	51.60 (45.92)	20.00 (26.55)	28.57 (32.29)	25.71 (30.45)	28.57 (32.29)	25.71 (30.40)	43.75 (41.40)	27.50 (31.61)	22.50 (28.30)	5.00 (12.91)	24.69 (28.56)	50.00 (45.00)	33.75 (35.50)	43.75 (41.40)	10.25 (18.66)	34.44 (35.14)
T_5 - Salicylic acid 25 ppm	66.66 (54.77)	87.85 (70.18)	100 (88.34)	100 (88.34)	88.63 (75.41)	48.57 (44.18)	67.14 (55.07)	78.57 (62.58)	28.57 (32.29)	56.07 (48.79)	30.00 (33.20)	25.00 (29.98)	15.00 (22.77)	10.00 (18.42)	20.00 (26.09)	35.00 (36.26)	36.25 (37.01)	30.00 (33.20)	15.25 (22.97)	29.13 (32.36)
T_6 - Salicylic acid 50 ppm	88.57 (70.91)	86.42 (68.83)	100 (88.34)	100 (88.34)	93.75 (79.11)	72.14 (65.25)	67.14 (55.07)	80.00 (63.62)	28.57 (32.29)	64.11 (53.80)	21.25 (27.43)	25.00 (29.98)	11.25 (19.58)	12.50 (20.69)	17.50 (24.42)	28.75 (32.41)	51.25 (45.71)	30.00 (33.20)	17.75 (24.90)	31.94 (34.06)
T_7 - Ascorbic acid 50 ppm	82.85 (65.80)	85.71 (68.19)	100 (88.34)	45.71 (42.53)	78.57 (66.22)	40.71 (39.63)	57.14 (49.11)	28.37 (32.17)	28.57 (32.29)	38.70 (38.30)	30.00 (33.20)	23.75 (29.15)	18.75 (25.64)	26.25 (30.80)	24.69 (29.70)	26.25 (30.80)	35.00 (36.26)	36.25 (37.01)	31.50 (34.13)	32.25 (34.55)
T_8 - Ascorbic acid 100ppm	85.71 (68.19)	82.85 (65.80)	100 (88.34)	45.71 (42.53)	78.57 (66.22)	40.71 (39.63)	46.42 (42.94)	28.57 (32.29)	28.57 (32.29)	36.07 (36.79)	25.00 (29.98)	30.00 (33.20)	18.75 (25.64)	5.00 (12.91)	19.69 (25.43)	26.25 (30.80)	53.33 (46.91)	36.25 (37.01)	10.25 (18.66)	31.52 (33.34)
T_9 - NAA 50 ppm	89.28 (71.66)	85.00 (67.58)	58.88 (50.13)	100 (88.34)	83.29 (69.43)	48.42 (44.09)	28.57 (32.29)	80.00 (63.62)	28.57 (32.29)	46.39 (43.08)	18.75 (25.64)	25.00 (29.98)	25.00 (29.98)	8.75 (17.19)	19.38 (25.70)	18.75 (25.64)	25.00 (29.98)	50.00 (45.00)	14.00 (21.96)	26.94 (30.65)
T_{10} - NAA 100 ppm	88.57 (70.91)	88.57 (70.91)	51.11 (45.63)	100 (88.34)	82.06 (68.95)	71.42 (64.69)	28.57 (32.29)	72.38 (58.37)	28.57 (32.29)	52.74 (46.91)	31.25 (33.97)	22.50 (28.30)	25.00 (29.98)	12.50 (20.69)	22.81 (28.24)	31.25 (33.97)	25.00 (29.98)	50.00 (45.00)	17.75 (24.90)	31.00 (33.46)
T_{11} - Distilled water	87.14 (69.50)	74.28 (59.62)	67.22 (55.12)	45.71 (42.53)	68.59 (56.69)	28.57 (32.29)	28.57 (32.29)	26.42 (30.92)	28.57 (32.29)	28.03 (31.95)	22.50 (28.30)	27.50 (31.61)	17.50 (24.71)	2.50 (9.09)	17.50 (23.43)	36.25 (37.01)	52.50 (46.43)	35.00 (36.26)	7.75 (16.15)	32.88 (33.96)
T_{12} - Control	61.42 (51.62)	82.14 (65.25)	66.11 (54.44)	45.71 (42.53)	63.85 (53.46)	28.57 (32.29)	28.57 (32.29)	24.28 (29.51)	28.57 (32.29)	27.50 (31.60)	31.25 (33.97)	30.00 (33.20)	15.00 (22.77)	21.25 (27.43)	24.38 (29.34)	31.25 (33.97)	47.50 (43.56)	30.00 (33.20)	26.50 (30.97)	33.81 (35.42)
Mean	75.03 (61.37)	78.45 (63.16)	79.80 (69.47)	72.86 (65.44)	76.53 (64.86)	43.72 (41.44)	43.63 (41.18)	47.26 (43.51)	28.57 (32.29)	40.80 (39.61)	26.15 (30.54)	26.25 (30.78)	17.40 (24.38)	11.35 (18.91)	20.29 (26.15)	30.83 (33.59)	40.07 (39.14)	35.75 (36.54)	16.60 (23.65)	30.81 (33.23)

(Values in parenthesis are arc sine transformed values)

	SED	CD 5%	CD 1%	SED	CD 5%	CD 1%	SED	CD 5%	CD 1%	SED	CD 5%	CD 1%
Packing (P)	0.90	1.80*	2.38**	0.55	1.11*	1.47**	0.21	0.43*	0.57**	0.38	0.76*	1.01**
Treatment (T)	1.57	3.11*	4.12**	0.96	1.92*	2.54**	0.37	0.75*	0.99**	0.67	1.33*	1.76**
Packing X Treatment (P X T)	3.14	6.23*	8.25**	1.93	3.84*	5.09**	0.75	1.50*	1.99**	1.34	2.66*	3.52**

Freshness index	Score
Almost all buds turgid	7
Partial to half open flowers, turgid	6
Half to full open flowers, turgid	5
Partial to half open flowers, slightly wilted	4
Half to full open flowers, slightly wilted	3
Partial to half open flowers, full wilted	2
Half to full open flowers fully wilted	1

Flower opening index	Score
Unopened buds	0
Slightly opened	1
Half opened	2
Full opened	3

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Table 2 : Effect of chemical treatments and different packaging material on Colour retention index (%) and fragrance of J. grandiflorum.

Packing / Treatment	Colour retention index (%)										Fragrance score							
	3 days after packing					6 days after packing					3 days after packing				6 days after packing			
	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ
T_1 - Sucrose 2%	88.88 (71.23)	84.44 (67.10)	100 (88.34)	100 (88.34)	93.33 (78.75)	60.00 (50.78)	66.44 (54.64)	88.88 (71.23)	44.44 (41.80)	64.94 (54.61)	3 3	3 3	3 3	3 3	2 2	2 2	2 2	2 2
T_2 - Sucrose 4%	88.88 (71.23)	88.88 (71.23)	100 (88.34)	100 (88.34)	94.44 (79.79)	44.44 (41.80)	44.44 (41.80)	88.88 (71.23)	44.44 (41.80)	55.55 (49.16)	3 3	3 3	3 3	3 3	2 2	2 2	2 2	2 2
T_3-Boric acid 2%	66.66 (54.77)	66.66 (54.77)	58.88 (50.13)	44.44 (41.80)	59.16 (50.37)	44.44 (41.80)	44.44 (41.80)	44.44 (41.80)	25.66 (30.42)	39.75 (38.95)	3 3	3 3	3 3	3 3	2 2	2 2	2 2	2 2
T_2 - Boric acid 4%	66.66 (54.77)	88.88 (71.23)	56.66 (48.83)	44.44 (41.80)	64.16 (54.16)	44.44 (41.80)	44.44 (41.80)	44.44 (41.80)	25.66 (30.42)	39.75 (38.95)	3 3	3 3	3 3	3 3	2 2	2 2	2 2	2 2
T_5 - Salicylic acid 25 ppm	88.88 (71.23)	88.88 (71.23)	100 (88.34)	100 (88.34)	94.44 (79.79)	50.00 (45.00)	83.33 (66.19)	88.88 (71.23)	44.44 (41.80)	66.66 (56.05)	3 3	3 3	3 3	3 3	2 2	2 2	2 2	2 2
T_6 - Salicylic acid 50 ppm	88.88 (71.23)	88.88 (71.23)	100 (88.34)	100 (88.34)	94.44 (79.79)	88.88 (71.23)	86.66 (69.05)	88.88 (71.23)	44.44 (41.80)	77.22 (63.33)	3 3	3 3	3 3	3 3	2 2	2 2	2 2	2 2
T_7 - Ascorbic acid 50 ppm	88.88 (71.23)	50.00 (45.07)	100 (88.34)	50 (45.00)	72.22 (62.39)	44.44 (41.80)	44.44 (41.80)	78.88 (71.23)	27.44 (31.57)	51.30 (46.60)	3 3	3 3	3 3	3 3	2 2	2 2	2 2	2 2
T_8 - Ascorbic acid 100 ppm	88.88 (71.23)	50.00 (45.07)	100 (88.34)	50 (45.00)	72.22 (62.39)	44.44 (41.80)	44.44 (41.80)	78.88 (71.23)	27.44 (31.57)	51.30 (46.60)	3 3	3 3	3 3	3 3	2 2	2 2	2 2	2 2
T_9 - NAA 50 ppm	88.88 (71.23)	88.88 (71.23)	66.66 (54.77)	100 (88.34)	86.11 (71.39)	50.00 (45.00)	86.44 (68.85)	44.44 (41.80)	44.44 (41.80)	56.33 (49.36)	3 3	3 3	3 3	3 3	2 2	2 2	2 2	2 2
T_{10} - NAA 100 ppm	50.00 (45.00)	88.88 (71.23)	66.60 (54.74)	100 (88.34)	76.37 (64.83)	77.77 (62.01)	84.44 (67.10)	44.44 (41.80)	44.44 (41.80)	62.77 (53.18)	3 3	3 3	3 3	3 3	2 2	2 2	2 2	2 2
T_{11} - Distilled water	88.88 (71.23)	80.00 (63.62)	95.00 (77.25)	44.44 (41.80)	77.08 (63.48)	22.22 (28.11)	22.22 (28.11)	83.33 (66.19)	25.77 (30.49)	38.39 (38.22)	3 3	3 3	3 3	3 3	2 2	2 2	2 2	2 2
T_{12} - Control	69.44 (56.50)	76.66 (61.24)	91.66 (74.71)	44.44 (41.80)	70.55 (58.56)	44.44 (41.80)	22.22 (28.11)	77.77 (62.01)	25.77 (30.49)	42.55 (40.60)	3 3	3 3	3 3	3 3	2 2	2 2	2 2	2 2
T_2 -Sucrose 2%	80.32 (65.07)	78.42 (63.67)	86.29 (74.21)	73.15 (65.50)	79.54 (67.14)	51.29 (46.08)	56.16 (49.25)	72.68 (60.23)	35.37 (36.31)	53.87 (47.97)								

(Values in parenthesis are arc sine transformed values)

		SED	CD 5%	CD 1%		SED	CD 5%	CD 1%
Packing (P)		1.04	2.07*	2.74**		0.87	1.73*	2.29**
Treatment (T)		1.80	3.58*	4.75**		1.51	3.00*	3.97**
Packing X Treatment (P X T)		3.61	7.17*	9.50**		3.02	6.01*	7.95**

Fragrance score

- Very strong - 4
- Strong - 3
- Mild - 2
- Least and undesirable - 1

Colour retention index

	Score		
Bright white	9	11 to 15% brown	5
Dull white	8	16 to 50% brown	4
Cream or yellowish	7	51 to 75% brown	3
1 to 10% brown	6	76 to 90% brown	2
		All brown	1

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Table 3 : Effect of chemical treatments and different packaging material on PLW and moisture content of *J. grandiflorum*.

Packing / Treatment	Physiological loss in weight (%)										Moisture content (%)									
	3 days after packing					6 days after packing					3 days after packing					6 days after packing				
	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean
T_1 - Sucrose 2%	1.67 (7.41)	0.61 (4.47)	0.00 (1.65)	0.00 (1.65)	0.57 (3.79)	2.58 (9.23)	2.14 (8.40)	1.29 (6.50)	3.62 (10.96)	2.41 (8.77)	50.00 (45.00)	45.6 (47.27)	56.05 (48.48)	56.16 (48.54)	51.95 (46.12)	40.16 (39.31)	23.68 (29.10)	31.85 (34.34)	25.14 (30.08)	30.21 (33.21)
T_1 - Sucrose 4%	1.67 (7.41)	1.36 (6.69)	0.00 (1.65)	0.00 (1.65)	0.76 (4.35)	3.45 (10.69)	2.21 (8.54)	3.68 (11.04)	3.45 (10.69)	3.20 (10.24)	37.32 (37.64)	40.79 (39.68)	56.12 (48.52)	56.51 (48.75)	47.69 (43.65)	25.99 (30.63)	24.76 (29.83)	31.49 (34.12)	26.13 (30.73)	27.09 (31.33)
T_3 - Boric acid 2%	2.61 (9.29)	2.14 (8.40)	2.14 (8.40)	2.71 (9.47)	2.40 (8.89)	3.57 (10.89)	3.33 (10.50)	3.89 (11.36)	3.87 (11.33)	3.67 (11.02)	26.79 (31.16)	31.46 (34.10)	32.11 (34.50)	25.76 (30.48)	29.0 (32.56)	25.84 (30.54)	22.93 (28.59)	25.96 (30.62)	25.88 (30.56)	25.15 (30.08)
T_4 - Boric acid 4%	2.68 (9.41)	2.14 (8.40)	2.19 (8.50)	2.71 (9.47)	2.43 (8.95)	4.00 (11.53)	3.20 (10.29)	3.71 (11.10)	3.29 (10.44)	3.55 (10.84)	25.99 (30.63)	31.97 (34.42)	31.87 (34.35)	25.54 (30.34)	28.84 (32.44)	23.18 (28.76)	24.87 (29.90)	25.43 (30.27)	25.64 (30.41)	24.78 (29.83)
T_5 - Salicylic acid 25 ppm	0.54 (4.19)	0.93 (5.52)	0.00 (1.65)	0.00 (1.65)	0.37 (3.25)	2.57 (9.22)	1.64 (7.35)	1.07 (5.94)	3.46 (10.71)	2.17 (8.25)	41.95 (40.36)	56.05 (48.48)	56.16 (48.54)	49.91 (44.94)	49.86 (44.90)	37.32 (37.64)	37.65 (37.84)	43.86 (41.46)	26.28 (30.82)	37.67 (37.76)
T_6 - Salicylic acid 50 ppm	0.57 (4.34)	0.68 (4.71)	0.00 (1.65)	0.00 (1.65)	0.31 (3.09)	1.09 (5.41)	1.64 (7.35)	1.00 (5.73)	3.55 (10.85)	1.79 (7.39)	49.56 (44.74)	56.11 (48.52)	56.19 (48.56)	48.75 (44.28)	52.65 (46.53)	35.67 (42.51)	37.78 (37.91)	44.80 (42.01)	26.10 (30.71)	38.35 (38.15)
T_7 - Ascorbic acid 50 ppm	0.86 (5.31)	0.71 (4.84)	0.00 (1.65)	2.71 (9.47)	1.07 (5.32)	2.96 (9.90)	2.14 (8.40)	3.58 (10.90)	3.82 (11.26)	3.13 (10.12)	46.38 (42.92)	48.02 (43.86)	55.12 (48.59)	25.59 (30.37)	44.06 (41.43)	22.97 (28.62)	24.18 (29.44)	31.26 (33.98)	25.98 (30.63)	26.10 (30.67)
T_8 - Ascorbic acid 100 ppm	0.71 (4.84)	0.86 (5.31)	0.00 (1.65)	2.71 (9.47)	1.07 (5.32)	2.96 (9.90)	2.68 (9.41)	3.66 (11.02)	3.19 (10.28)	3.12 (10.15)	47.46 (43.54)	46.38 (42.92)	55.02 (48.64)	26.13 (30.73)	44.07 (41.45)	22.80 (28.51)	21.00 (27.26)	25.88 (30.56)	25.67 (30.43)	23.84 (29.19)
T_9 - NAA 50 ppm	0.54 (4.19)	0.75 (4.96)	2.06 (8.24)	0.00 (1.65)	0.84 (4.76)	2.58 (9.23)	3.57 (10.89)	1.00 (5.73)	3.37 (10.57)	2.63 (9.10)	49.82 (44.89)	42.00 (40.39)	27.37 (31.53)	56.18 (48.56)	43.84 (41.34)	40.11 (39.28)	28.00 (31.93)	44.18 (41.65)	26.43 (30.92)	34.68 (35.95)
T_{10} - NAA 100 ppm	0.57 (4.34)	0.57 (4.34)	2.44 (8.98)	0.00 (1.65)	0.90 (4.83)	0.93 (5.52)	3.49 (10.76)	1.38 (6.74)	3.49 (10.76)	2.32 (8.44)	45.60 (42.47)	42.86 (40.88)	38.62 (38.41)	56.31 (48.63)	45.85 (42.60)	44.36 (41.75)	40.35 (39.42)	27.96 (31.91)	25.66 (30.42)	34.58 (35.88)
T_{11} - Distilled water	0.64 (4.58)	1.29 (6.50)	1.64 (7.35)	2.71 (9.47)	1.57 (6.98)	3.64 (10.99)	3.67 (11.03)	3.68 (11.04)	3.68 (11.05)	3.67 (11.03)	48.72 (44.26)	41.95 (40.36)	37.64 (37.83)	26.47 30.95)	38.70 (38.35)	26.00 (30.64)	21.79 (27.81)	20.16 (26.66)	25.83 (30.53)	23.45 (28.91)
T_{12} - Control	1.93 (7.97)	0.89 (5.41)	1.69 (7.47)	2.71 (9.47)	1.81 (7.58)	3.26 (10.39)	3.70 (11.08)	3.79 (11.21)	3.50 (10.77)	3.56 (10.86)	34.93 (36.21)	45.67 (42.51)	37.02 (37.46)	25.00 (29.98)	35.66 (36.54)	26.11 (30.71)	22.95 (28.61)	20.41 (26.84)	25.71 (30.45)	23.80 (29.15)
Mean	1.25 (6.11)	1.08 (5.80)	1.01 (4.90)	1.36 (5.56)	1.17 (5.59)	2.78 (9.41)	2.78 (9.50)	2.64 (9.03)	3.52 (10.81)	2.93 (9.69)	41.66 (40.09)	42.95 (40.90)	45.14 (42.12)	40.98 (39.53)	42.68 (40.66)	28.65 (32.25)	29.95 (32.92)	32.10 (34.30)	25.87 (30.56)	29.14 (32.51)

(Values in parenthesis are arc sine transformed values)

	SED	CD 5%	CD 1%	SED	CD 5%	CD 1%	SED	CD 5%	CD 1%	SED	CD 5%	CD 1%
Packing (P)	0.05	0.11*	0.15**	0.09	0.18*	0.24**	0.64	1.28*	1.69**	1.08	2.14*	2.83**
Treatment (T)	0.10	0.20*	0.26**	0.16	0.32*	0.42**	1.12	2.22*	2.94**	1.87	3.71*	4.91**
Packing X Treatment (P X T)	0.20	0.40*	0.53**	0.32	0.64*	0.85**	2.24	4.44*	5.88**	3.74	7.43*	9.83**

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Table 4 : Effect of chemical treatments and different packaging material on RWC and membrane integrity of J. grandiflorum.

Packing / Treatment	Relative Water Content (%)										Membrane Integrity (%)									
	3 days after packing					6 days after packing					3 days after packing					6 days after packing				
	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean	P_1 20 µ	P_2 40 µ	P_3 60 µ	P_4 80 µ	Mean
T_1 - Sucrose 2%	80.99 (64.36)	73.87 (59.35)	90.80 (73.47)	90.98 (73.71)	84.16 (67.72)	65.06 (53.80)	38.36 (38.26)	51.60 (45.91)	40.73 (39.64)	48.94 (44.40)	81.48 (64.74)	65.12 (53.83)	57.14 (49.11)	57.14 (49.11)	65.18 (54.18)	73.60 (50.30)	93.87 (75.79)	83.82 (66.59)	92.08 (75.45)	85.84 (67.03)
T_2 - Sucrose 4%	60.46 (51.05)	66.08 (54.42)	90.91 (73.62)	91.55 (74.53)	77.25 (63.40)	42.10 (40.45)	40.11 (39.28)	51.01 (45.58)	42.33 (40.58)	43.89 (41.47)	81.48 (64.74)	76.97 (61.45)	57.04 (49.06)	56.54 (48.76)	68.01 (56.00)	91.03 (59.39)	92.55 (76.73)	84.27 (66.96)	90.86 (73.55)	89.68 (69.16)
T_3 - Boric acid 2%	43.40 (41.20)	50.97 (45.55)	52.02 (46.16)	41.73 (40.23)	47.03 (43.28)	41.86 (40.30)	37.15 (37.54)	42.06 (40.42)	41.93 (40.34)	40.75 (39.65)	95.17 (77.49)	89.10 (71.46)	88.26 (70.59)	96.51 (81.73)	92.26 (75.32)	91.22 (59.58)	94.80 (76.92)	91.07 (73.82)	91.17 (73.97)	92.07 (71.07)
T_4 - Boric acid 4%	42.10 (40.45)	51.79 (46.02)	51.63 (45.93)	41.37 (40.02)	46.72 (43.11)	37.55 (37.78)	40.29 (39.39)	41.20 (39.92)	41.54 (40.12)	40.15 (39.30)	96.21 (79.05)	88.44 (70.77)	88.57 (70.90)	96.80 (80.07)	92.51 (75.20)	94.49 (65.50)	92.41 (76.22)	91.72 (74.81)	91.46 (74.40)	92.52 (72.73)
T_5 - Salicylic acid 25 ppm	60.46 (51.05)	80.85 (64.26)	90.98 (73.71)	90.80 (73.47)	80.77 (65.62)	60.99 (51.37)	67.96 (55.57)	72.58 (58.50)	42.57 (40.72)	61.03 (51.54)	65.00 (53.76)	70.72 (57.31)	56.99 (49.03)	57.01 (49.03)	62.46 (52.30)	71.40 (57.74)	76.69 (61.26)	69.05 (56.25)	90.68 (73.31)	76.67 (61.96)
T_6 - Salicylic acid 50 ppm	80.29 (63.84)	78.98 (62.87)	91.03 (73.77)	90.90 (73.60)	85.30 (68.52)	61.20 (51.49)	71.57 (57.85)	73.99 (59.43)	42.28 (40.55)	62.13 (52.25)	65.57 (54.11)	66.63 (54.75)	56.95 (49.00)	57.06 (49.06)	61.55 (51.73)	69.83 (54.87)	76.53 (61.15)	67.90 (55.53)	90.90 (73.60)	75.83 (61.47)
T_7 - Ascorbic acid 50 ppm	75.14 (60.20)	77.79 (62.03)	89.01 (73.89)	41.46 (40.07)	71.38 (59.05)	37.21 (37.58)	39.17 (38.73)	50.64 (45.36)	42.09 (40.44)	42.28 (40.53)	69.71 (56.66)	67.57 (55.33)	56.89 (48.97)	91.04 (73.79)	71.30 (58.69)	94.75 (76.91)	93.26 (75.06)	84.55 (67.19)	96.73 (79.94)	92.32 (74.78)
T_8 - Ascorbic acid 100 ppm	76.89 (61.40)	75.14 (60.20)	89.24 (74.07)	42.33 (40.58)	71.40 (59.06)	36.94 (37.41)	34.02 (35.67)	41.93 (40.34)	41.59 (40.15)	38.62 (38.39)	68.30 (55.79)	69.71 (56.66)	56.78 (48.91)	91.43 (74.34)	71.56 (58.92)	94.96 (77.20)	97.17 (80.79)	91.17 (73.97)	96.03 (78.76)	94.83 (77.68)
T_9 - NAA 50 ppm	80.71 (64.15)	68.04 (55.62)	44.34 (41.74)	91.01 (73.75)	71.03 (58.82)	64.98 (53.75)	71.05 (57.52)	45.36 (42.33)	42.82 (40.86)	56.03 (48.61)	65.23 (53.90)	75.40 (60.38)	94.42 (76.47)	56.97 (49.01)	73.01 (59.94)	73.66 (59.21)	88.56 (70.89)	68.66 (56.01)	90.49 (73.07)	80.47 (64.80)
T_{10} - NAA 100 ppm	73.87 (59.35)	69.43 (56.49)	62.56 (52.30)	91.22 (74.04)	74.27 (60.55)	45.30 (42.29)	65.37 (53.99)	71.86 (58.04)	41.57 (40.13)	56.18 (48.70)	70.72 (57.31)	74.28 (59.62)	79.79 (63.47)	56.80 (48.91)	70.40 (57.33)	69.44 (55.87)	88.61 (70.95)	73.37 (59.02)	91.44 (74.36)	80.34 (65.05)
T_{11} - Distilled water	78.93 (62.84)	67.96 (55.57)	60.98 (51.36)	42.88 (40.90)	62.69 (52.67)	42.12 (40.46)	35.30 (36.44)	32.66 (34.84)	41.84 (40.29)	37.98 (38.01)	66.66 (54.77)	75.47 (60.42)	81.07 (64.42)	91.23 74.06)	78.61 (63.42)	91.02 (73.76)	96.20 (79.04)	98.20 (82.51)	95.59 (78.09)	95.25 (78.35)
T_{12} - Control	56.59 (48.79)	73.99 (59.43)	59.97 (50.77)	40.50 (39.51)	57.76 (49.62)	42.30 (40.56)	37.18 (37.56)	33.06 (35.09)	41.65 (40.18)	38.55 (38.35)	84.59 (67.23)	70.63 (57.25)	81.87 (65.04)	91.38 (74.27)	82.12 (65.95)	90.88 (73.58)	94.77 (76.94)	97.90 (82.72)	97.50 (81.53)	95.26 (78.68)
Mean	67.49 (55.72)	69.57 (56.82)	73.13 (60.90)	66.39 (57.03)	69.15 (57.62)	46.42 (42.91)	48.51 (44.23)	51.99 (46.26)	41.91 (40.33)	47.21 (43.43)	75.84 (61.63)	74.17 (59.94)	71.31 (58.75)	74.99 (62.68)	74.08 (60.75)	87.76 (71.07)	86.17 (70.36)	83.52 (63.66)	92.91 (75.83)	87.59 (70.23)

(Values in parenthesis are arc sine transformed values)

	SED	CD5%	CD1%		SED	CD5%	CD1%		SED	CD5%	CD1%		SED	CD5%	CD1%
Packing (P)	1.18	2.34*	3.11**		0.55	1.09*	1.45**		0.64	1.28*	1.69**		1.08	2.14*	2.83**
Treatment (T)	2.05	4.06*	5.38**		0.95	1.90*	2.51**		1.12	2.22*	2.94**		1.87	3.71*	4.91**
Packing X Treatment (P X T)	4.10	8.13*	10.7**		1.91	3.80*	5.03**		2.24	4.44*	5.88**		3.74	7.43*	9.83**

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Table 5 : Effect of chemical treatments and different packaging material on shelf life of *J. grandiflorum*.

Packing / Treatment	Shelf life in hours				
	P_1 20 u	P_2 40 u	P_3 60 u	P_4 80 u	Mean
T_1 - Sucrose 2%	120.22	120.34	144.20	120.32	126.27
T_2 - Sucrose 4%	96.32	96.22	144.26	120.22	114.26
T_3 - Boric acid 2%	96.44	96.46	120.18	96.34	102.36
T_4 - Boric acid 4%	96.62	96.12	120.20	96.26	102.30
T_5 - Salicylic acid 25 ppm	120.26	144.22	156.28	132.42	138.33
T_6 - Salicylic acid 50 ppm	148.32	144.64	156.32	132.56	150.43
T_7 - Ascorbic acid 50 ppm	96.44	96.40	120.24	96.18	102.32
T_8 - Ascorbic acid 100 ppm	96.24	96.20	120.28	96.32	102.26
T_9 - NAA 50 ppm	120.22	120.14	120.56	120.22	120.29
T_{10} - NAA 100 ppm	148.24	120.26	120.22	120.40	132.28
T_{11} - Distilled water	96.34	96.34	120.46	96.42	102.39
T_{12} - Control	96.46	96.42	120.12	96.22	102.31
Mean	114.34	112.29	128.30	110.32	116.31

		SED	CD 5%	CD 1%	
Packing (P)		2.23	4.44*	5.88**	
Treatment (T)		3.87	7.69*	10.18**	
Packing X Treatment		7.75	15.39*	20.37	

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Isolation of New Phosphate Solubilizer *Pseudomonas fluorescens* SSC from Doon Valley

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The present study was conducted at Botany Department, D. A. V. (P.G.) College, Dehradun with a view to isolate and characterize the bacteria present in the agriculture soil of Doon Valley. In total, four soil samples were collected from different locations such as Sahaspur, Raipur, Doiwala and Majra of Doon Valley. Isolation of the bacterial strains was done by dilution plate technique followed by three-tier screening process by doing morphological studies, physiological studies and biochemical characterization. The identification of strain was done by Institute of Microbial Technology, Chandigarh, India and was named as *Pseudomonas fluorescens* SSC from collected soil sample. The strain was further screened for their role in P solubilization in laboratory to promote it as PSB in agriculture soil.

Keywords : PSB- phosphate solubilizing bacteria; P- phosphate.

INTRODUCTION

Soils generally contain adequate amount of inorganic and organic phosphorous but most of it remain unavailable to plants. The efficiency of water soluble phosphorous is usually low and its recovery does not exceed 20% because P when added to soil forms $Ca_3(PO_4)_2$ in calcareous and alkaline soils and $FePO_4$ and $AlPO_4$ in acidic soils. In Doon valley, soil is rich in P but it gets fixed in soil. Therefore in the present study, an attempt has been made to isolate a bacterial strain prevalent in agriculture soil of doon valley and evaluating their morphological characters. The isolate was further subjected to various physiological and biochemical characterization procedures with a view to identify them. The native strain would be screened for their potential role in solubilizing P in soil to apply as biofertilizer.

MATERIAL AND METHODS

Study area : Doon valley lies in the foothills of Himalayas and is located in 30° 20' N latitude and 78° 04' E longitude. The valley possesses sub-tropical climate. The maximum temperature during summer reaches upto 36°C and minimum upto 4°C during winters. The average rainfall is 2051.4 mm and mostly occurs from June to September and little rain occurs in winters also. These conditions makes Doon valley favourable for agriculture practices.

Collection of soil sample : Altogether four soil samples from agriculture fields were collected from Sahaspur, Raipur, Doiwala and Majra locations of Doon valley by digging upto 5-15 cm under the soil after removing the litter from the top, in polythene bags and stored at room temperature. Finally, it was air dried in shade and ground to pass through 2 mm sieve. Sufficient amount of sieved soil of each sample was kept in clean and sterilized bags for isolation.

Isolation from soil samples : Isolation of native microorganisms present in the soil was done by dilution plate technique using nutrient agar medium. Three replica plates were also prepared for each sample. The plates were incubated at 28 + 2 °C for 24 - 48 h.

Screening of phosphate solubilizing fluorescent *Pseudomonas* strains (Subba Rao, 1982) : Different types of bacterial colonies with characteristic colour were obtained by serial dilution and plating on Pikovskaya's media. Each strain of bacterium was spot inoculated on Pikovskaya's media plate containing tricalcium phosphate (TCP) separately and incubated for 4-5 days at 28 ± 2 °C to observe the clear or halo zone around the colony. The strains showing zone of solubilization were presumed to be P solubilizers which were then subjected to culture on King's B media.

Characterization of fluorescent bacteria : After purification of bacterial colonies on King's B medium, the bacterial colony was examined on the basis of morphology, physiology and biochemical tests (Holt *et al.*, 1994; Cappuccino, 2006).

Morphological characteristics : The colony characteristics were studied on the basis of shape, colour and structure.

Physiological studies : The isolated strain was streaked on nutrient agar (NA), Pikovskaya's media (PVK) and King's B media (King's *et al.*, 1954) respectively and the growth of bacterial colony was studied.

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Biochemical characterization : Various biochemical tests were carried out to characterize the isolated bacterial strain. The biochemical tests include pigmentation test, motility test, oxidase, catalase, indole test, methyl red (MR) and Voges proskrauer (VP) test, citrate utilization test, gelatin hydrolysis test, for gelatin starch and urea fermentation test and triple sugar iron test (TST).

RESULTS

Isolation : Number of probable isolates were isolated from the prepared soil samples by following the method of Subba Rao (1982). The soil of Raipur and Sahaspur shows the presence of fluorescent *Pseudomonas* strain. The colonies were screened on the basis of zone formation by isolates on Pikovskaya's TCP media plate.

Characteristics of isolate : Morphological characterization was done by Gram's staining to differentiate between two principal groups of bacteria- Gram positive and Gram negative and examined the shape of microorganism. The isolate was found Gram negative rod and contains more than one flagella. During physiological studies, isolate on different media shows different growth patterns. On nutrient agar media (NAM), *P. fluorescens* colony appeared yellow in color. On Pikovskaya's modified media containing TCP a clear zone was observed around the colony of isolate after incubation of 5 days. The isolate also decolorizes the Pikovskaya's broth, the characteristic of *Pseudomonas* bacteria. But on King's B media (KBM) *P. fluorescens* shows good growth and appears white in color and under light it shows fluorescence.

Further, the isolated strain was named as *Pseudomonas fluorescens* SSC by Institute of Microbial Technology (IMT), Chandigarh, India. Stock culture of *P. fluorescens* SSC strain was maintained in 20% glycerol at -70 °C. Biochemical characterization of *P. fluorescens* SSC was done in accordance to (Stanier *et al.*, 1966) and results are presented in Table 1.

***In vitro* phosphate solubilization (qualitative) by *P. fluorescens* SSC :** The test organism forms a clear or halo zone around the colony of bacteria on Pikovskaya's media (Pikovskaya, 1948) containing tricalcium phosphate. The zone of phosphate solubilization on 7th day and 15th day was 16 mm and 33 mm as shown in Photo (A, B). This indicates that the isolate was able to solubilize P.

DISCUSSION

On the basis of the observations of the isolate from Doon valley, it was identified by Institute of Microbial Technology (IMT), Chandigarh, India and named as "*Pseudomonas fluorescens* SSC". It is a Gram-negative rod-shaped bacteria and synthesizes blue-green fluorescent pigment in Pikovskaya's broth containing TCP due to the presence of pyocyanin pigment, the characteristic of *Pseudomonas* spp. Besides other activities, SSC shows the ability to ferment sucrose and partly to mannitol but unable to ferment lactose.

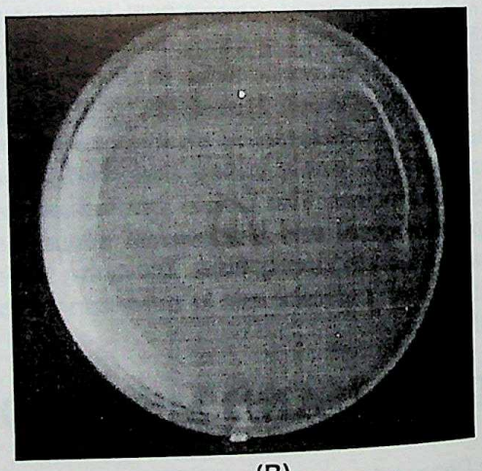
Table 1 : Characteristics of *Pseudomonas fluorescens* SSC species.

Characters	*P. fluorescens* SSC
Flagella	>1
Pigment production	gb
Catalase activity	+
Gelatin hydrolysis	+
Urea hydrolysis	-
Fermentation of Sucrose	+
Mannitol	D
Lactose	-
Indole production	+
MR-VP test	-
Citrate utilization	+
Oxidase reaction	+
Starch hydrolysis	+
Hydrogen sulphide production	-

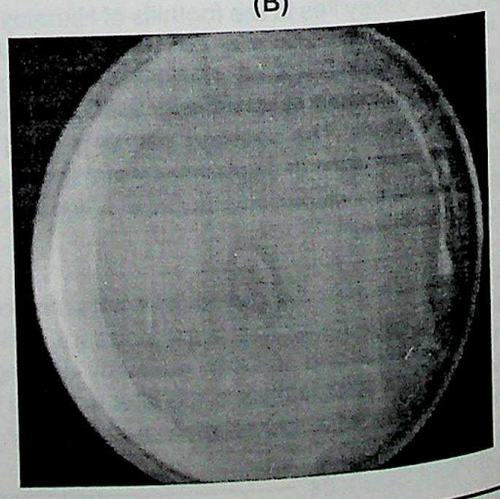
Abbreviations: gb, green blue; +, positive; -, negative; D, 70-80% positive

Photo I : Zone of phosphate solubilization by *P. fluorescens* SSC on (A) 7th day (B) 15th day on Pikovskaya's TCP media plate.

(A)



(B)



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These characters of *P. fluorescens* SSC resembles to those done earlier (Stanier *et al.*, 1966; Holt *et al.*, 1994; Dhal and Thatoi, 2007). The strain shows better plant growth promoting activity such as P solubilization by forming a clear zone around the colony on Pikovskaya's media containing TCP (Subba Rao, 1982; Anand *et al.*, 2007). The isolated PSM results in decolorization of Pikovskaya's broth, the characteristic of an efficient P solubilizer (Subba Rao, 1982). It reduces the pH of TCP broth upon incubation and shows maximum growth on King's B media as compared to nutrient agar and Pikovskaya's TCP media (Dave and Patel, 1999). Thus it can be concluded that the new isolate "*Pseudomonas fluorescens* SSC" is able to solubilize phosphate, an important plant growth promoting activity and it can be promoted as PSB in agriculture fields. However, further evaluation of microbe is required for such roles in laboratory and field conditions for exploitation in agriculture.

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Biochemical Estimations in Fern Cultivars Exposed to UV-B Radiations

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Three species of *Cheilanthes* (*C. rufa, C. tenuifolia* and *C. farinosa*) were chosen for studying the modulation in biochemical parameters in UV-B treated plants. UV radiations at very low doses were found to show various deleterious effects. Dry seeds showed no/little abnormalities. Growth measured in terms of total protein contents showed the maximum amount in *Cheilanthes farinosa* (45 µg/ml) while % reduction in protein contents was 15.56% at the 60 minutes exposure of UV-B radiations. Total amount of chlorophyll was affected by UV-B exposure in all three species of *Cheilanthes.* Maximum reduction was observed in *Cheilanthes rufa* followed by *Cheilanthes tenuifolia* and *Cheilanthes farinosa. Cheilanthes farinosa* showed the maximum contents of non-enzymatic antioxidants- ascorbate and proline. The *Cheilanthes farinosa* showed the maximum antioxidant potential in comparison with *Cheilanthes rufa* and *Cheilanthes tenuifolia* against UV-B stress.

Keywords : Free radicals, Proline, Ascorbate, Pigments, *Cheilanthes* spp.

INTRODUCTION

Ferns and their allies grow in many different habitats around the world. They were at their height during the Carboniferous Period (the age of ferns) as they were the dominant part of the vegetation at that time. During this era some fern like groups actually evolved seeds (the seed ferms) making up perhaps half of the fern like foliage in Carboniferous forests and much later gave rise to the flowering plants (Antony *et at.*, 2000). *Cheilanthes* species have a lip-like false indusium covering the sporangia. Its rhizome are usually erect to long-creeping, scales brown to black or often bicolored with dark central stripe and frond: evergreen, monomorphic, hairy, scaly or glabrous. The plants prefer light (sandy), medium (loamy) and heavy (clay) soils (Hitchcock *et al.* 1969; Hevly *et al.*, 1963).

Several anthropogenic human activities have led to the release of hydrocarbons and many trace gases in the atmosphere resulting in the depletion of ozone layer. Such depletion is expected to increase the amount of solar UV-B (280-320nm), which can damage the biological ecosystems. Numerous studies have been conducted on the effects of enhanced UV radiations on photosynthetic enzymes, pigments (Heath and Packer, 1968), proteins, seed patterns and antioxidant compound contents in plants. Hence the authors were interested to see the effect of U-B irradiations on different *Cheilanthes* species. The presence/absence and increasing/decreasing property of antioxidant compounds, production of free radicals and the amount of lipid peroxidation in terms of total MDA contents, might provide significant clues to assess and evaluate the antioxidant potential of various *Cheilanthes* species against environmental stresses (Archer, 2000). Furthermore, many groups of stresses like heavy metals, ultraviolet radiations etc are shown to generate singlet oxygen and other active oxygen species at various sites of photosynthetic electron transport chain (Halliwell and Gutterridge -1994) and affect the growth of plant. Considering the importance of *Cheilenthes* in the ecosystem and their capacity to protect itself under stress conditions; the authors have set forth the objective of investigating the growth pattern, antioxidant system, pigment content and protein profiles of three species of *Cheilenthes* (*C.rufa, C.tenuifolia* and *C. farinosa*), grown at different exposures of UV-B radiations.

MATERIAL AND METHODS

Organisms and culture conditions : Spores of *C. rufa, C. tenuifolia* and *C. farinosa* were collected from plants growing in the kushmi forest of Gorakhpur (a tarai area of north India). The spores stored in desiccators were surface sterilized with 2% sodium hypochlorite solution and then sown uniformly on 25 ml of autoclave sterilized (15 1b/in^2) inorganic medium at pH 5.4 in petri dishes (Sheffield *et al.*, 2001). Spores were subjected to irradiation at 87 ergs/mm^2/sec intensity by a 15 watt Philips germicidal lamp having a peak output of 253 nm at a distance of 8 inch from the UV source. Each experiment was conducted in triplicates.

Growth and photosynthetic pigments measurement : Growth was measured in terms of total protein contents using Folins-Lowry method with lysozyme as the standard (Lowry *et al.*, 1951). Total amount of chlorophyll from each sample of plant leaves were extracted in 80% acetone and the contents of pigments were determined by taking absorbance at 663 nm using UV-Vis spectrophotometer (Ultrospec-4000, USA).

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Proline and Ascorbate estimations : Proline contents in leaf homogenate of UV-B treated and untreated cultures were estimated according to the method of Bates *et al.,* (1973). Amount of proline is represented in terms of µg g^{-1} FW. Ascorbate was extracted and estimated by using the method of Show and Zilva (1943) using L-Ascorbate as standard.

RESULTS AND DISCUSSION

The spores of *C. farinosa* germinated after four days of sowing with 98% germinability, whereas those of *C. tenuifolia* and *C. rufa* germinated after 5 - 6 days with 95% and 93% germinability, respectively. The rate of cell division and elongation decreased with the increase of the dose. The average protonemal length on 20th day of germination in control cultures were 635.19 µm, 648.25 and 637.85 µm, in *C. rufa*, *C. tenuifolia* and *C. farinosa,* respectively. Among the irradiated cultures, values were getting reduced with increase of duration of doses (Figure 1). Such reduction in the length of protonema might be due to the impairment of cell division because of mitotic arrest at higher doses dose of irradiation. Similar findings are reported by other authors also (Ashcroft & Sheffield, 2000; Knobloch, 1969; Raghavan, 1980). Exposure to UV for 60 minutes resulted in the death of germinating spores. Low doses (20 and 40 min) had different apparent effects showing abnormal rhizoid differentiation such as bulbous and chlorophyllous rhizoids. Both the number and length of rhizoids decreased as the UV exposure increased.

Generally, *Cheilanthes* species contain appreciable amount of the basic food nutrients; protein, fats and carbohydrates (Cuming, 1999). Total protein contents were analysed to diversity different *Cheilanthes* cultivars. Protein contents were found to be notably variable in all three species of *Cheilanthes*. In control cultures, the total amount of protein contents were shown to be 35, 42, 45 µg/ml in *C. rufa*, *C. tenuifolia* and *C. farinosa*, respectively. Initially protein contents were found to be increasing with the increased doses of UV-B radiations that in turn decreased in all three species. The initial increase in amounts may be due to the increased activity of enzymatic antioxidants that help the plants in overcoming stress conditions. % decrease in protein contents was found to be maximum in *Cheilanthes rufa* followed by *C. tenuifolia and C. farinosa,* respectively (Figure 2 A). Total amount of pigments were maximum in *Cheilanthes farinosa* (5.8 mg pigment g^{-1} FW), and minimum in *Cheilanthes rufa* (4.3 mg pigment g^{-1} FW) (Figure 2 B). The high content of photosynthetic pigments in *Cheilanthes farinosa* might be responsible for the high growth in this plant, thus increasing the fresh mass and height of the plants. Pigments contents showed hig sensitivity towards UV exposures. Many-authors have reported the same extent of pigment contents, before and confirm the result findings, in the present work (Gratani *et al.,* 1998).

Measurement of Ascorbate and proline accumulation is an important criteria for determination of antioxidant capacity of plants exposed to any stress condition like drought, UV-rediations, heavy metals, herbicides etc. *Cheilanthes* accessions grown in the similar habitats were estimated for these compounds. The contents of such non-enzymatic antioxidants might give suitable keys for the biodiversity assessment in various accessions of *Cheilanthes* species. The contents of proline were measured in different species (Figure 3 A). The higher concentration was found to be in *C. farinosa* (7 µg g^{-1} FW) and least in *Cheilanthes rufa* (4 µg g^{-1} FW). It was observed that proline was being accumulated in leaves at low exposures of UV radiations, in all three species. The accumulation of compounds such as proline in higher plant cells in response to environmental strees is well documented before proving the present study (Cechin *et al.,* 2006; Aloni and Rosenshtein 1984). Proline provides less than 5% of the total pool of free amino acids in plants under strees free condition, whereas the concentration increased up to 80% of the amino acid pool during stress (Matysik *et al.,* 2002). The function of Proline in stressed plants is often explained by its property as an osmolyte, able to balance water stress (Delauney and Verma, 1993; Singh *et al.*, 1973). In addition possible positive roles of proline under stress have been proposed which includes stabilization of proteins (Anjum *et al.,*2000), scavenging of hydroxyl radicals (Smirnoff and Cumbes, 1989) and regulation of NAD/NADH ratio. Proline protects plants against singlet oxygen and free radical induced damages. Beside proline, ascorbic acid also serves as an important antioxidant metabolite with several other important cellular functions. Figure 3 B shows the various concentrations of ascorbate in young leaves of different *Cheilanthes* species. The ascorbate contents ranged from 25-68 (µg g^{-1} FW). The highest concentration was observed in *Cheilanthes farinosa* and least in *Cheilanthes rufa* Initial increase in values in ascorbate in *Cheilanthes rufa* and *Cheilanthes tenuifolia* were observed with the increase in UV-exposure. Later on the values were found to be decreasing at high doses. Though the values were always more than untreated cultures. The ascorbic acid is one of the most studied and powerful antioxidant (Horemans *et al.,* 2000). It has been detected in majority of the plant cell types, organelles and in the apoplast, Under physiological conditions, ascorbic acid exists mostly in the reduced form in leaves and chloroplasts (Smirnoff, 2000). The ability to donate electrons in a wide range of enzymatic and non-enzymatic reactions makes ascorbic acid the main AOS detoxifying cmpound in the aqueous phase. Ascorbic acid can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and reduce H_2O_2 to water via ascorbate peroxidase reaction (Noctor and Foyer, 1998). In addition, ascorbic acid has been implicated in the regulation of the cell division, cell progression from G_1 to S phase.

Cheilanthes species are good Phytoremediators and can be used remove heavy metals from the soil. Plants are grown in polluted soil. It is seen that the injury caused by oxygen species produced because of different stresses, in plants, might be suppressed by supplying antioxidants exogenously during exposure of plants to various physical stresses. These compounds include ascorbic acid, glutathione, proline, tocopherol, sodium benzoate, benzoquinone, DMTU (demethyl thio urea), carotenoids etc. Such compounds have been shown to reduce the extent of oxidative damage. In present study, it was proved that *Cheilanthes farinosa* has more antioxidant

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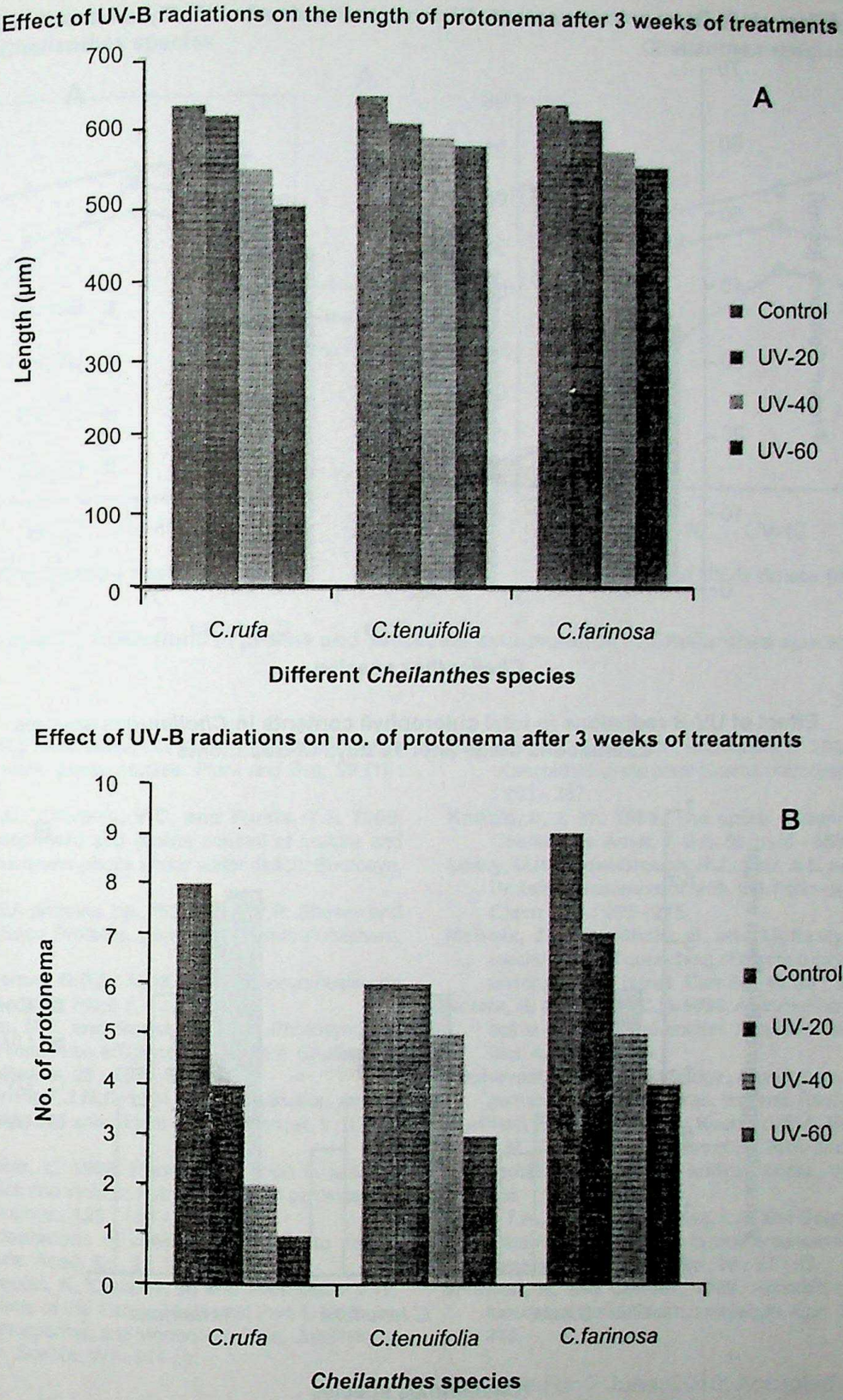

Figure 1 : Effect of UV-B exposures on protonemal growth in *Cheilanthes* cultivars

potential than other two species and this study can be used not only for the assessment of diversity in these species of *Cheilanthes* but also provide suitable models for the studies on UV-B stresses and antioxidant systems.

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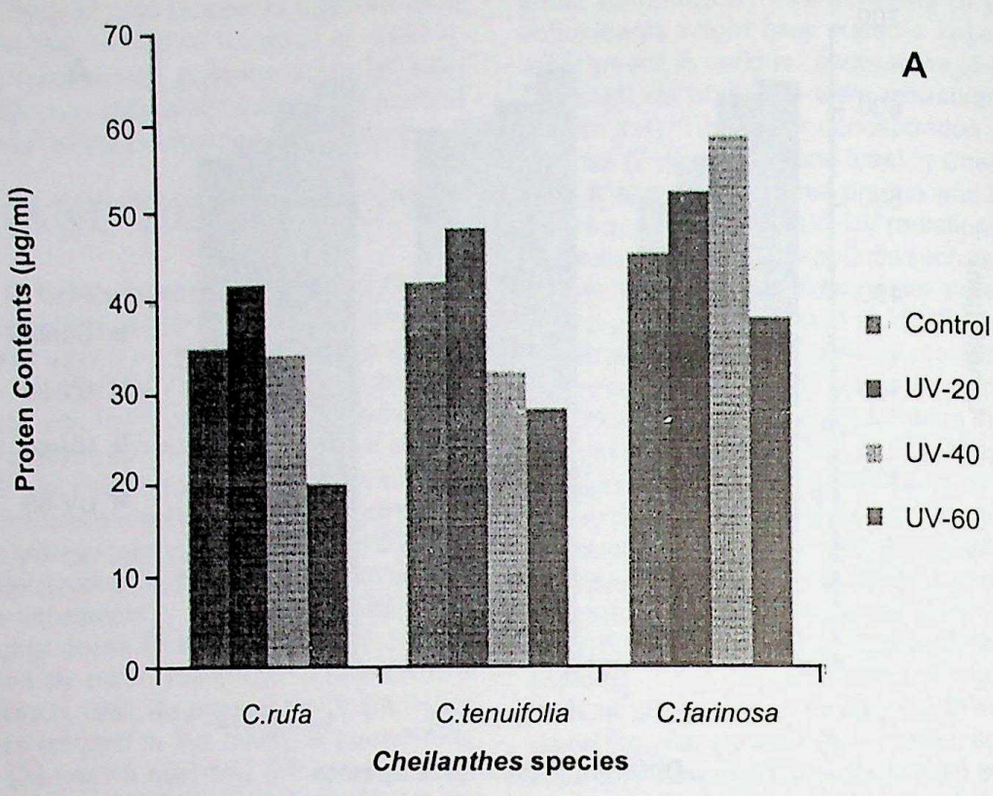
Effect of UV-B on protein contents *Estimations made after 10 days of treatments



Effect of UV-B radiations in total chlorophyll contents in *Cheilanthes* species *Estimations made after 10 days of treatments

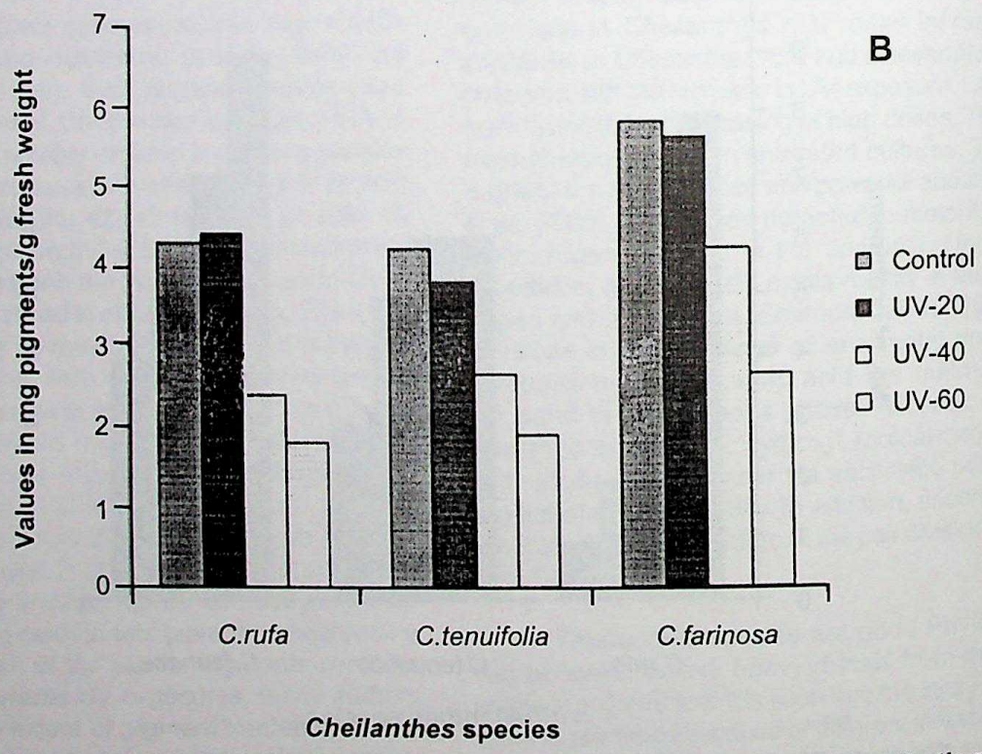


Figure 2 : Effect of the stress on growth (in terms of total protein contents) and photosynthetic pigments

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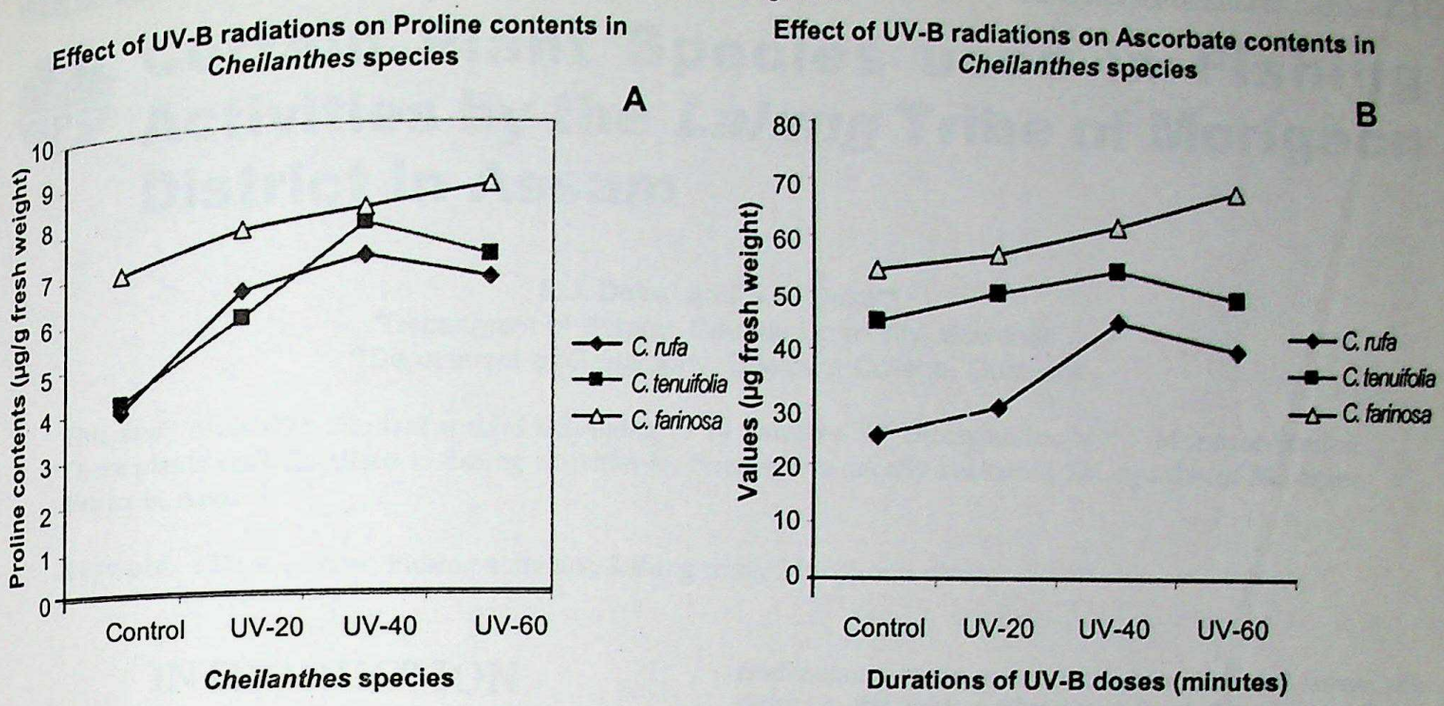

Figure 3 : Alterations in proline and ascorbate accumulation in *Cheilanthes* species

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Certain Plant Species used in Fishing Activities by the *Lalung* Tribe of Morigaon District in Assam

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This study deals with 20 plant species belonging to 14 Families, 13 Dicotylendon and 7 Monocotylendon. These plants are being used in fishing activities by Socio-economically backward *Lalung* tribe of Morigaon district in Assam.

Keywords : Plant species, Fishing activities, Lalung tribe, Morigaonn district.

INTRODUCTION

The tribe *Lalung* are estimated of about 0.1 million, mainly in the plains of Assam in northeast India. They are a branch of the Boro race. They prefer to call themselves as *Tiwa* and believe in a supreme deity *Lord Mahadeva* address as *"Fa Mahadeva"*. The social structure of the *Lalungs* is Primarily Patriarchal. The *Bihu* festival of Assam is the principal festival of the agrarian *Lalungs*. They celebrated the *Bihu* along with other peoples of Assam with a great joy and have traditionally caught fishes in the nearby wetlands which are locally known as *beel* with the neighboring villagers especially on *Bihu* festivals and other social occasions. Rice and fish is their delicious dish call *Bhatt-mass*. Fish is an essential food item of the *Lalungs*. The *Lalung* (*Tiwa*) tribe of Morigaon district in Assam used certain local growing plant species in their fishing activities. Fishing is not only a hobby of them but also related to their socio-cultural behavior. Through fishing they procure required amount of fish for their home consumption and store as dry fish for future consumption from the wetlands or beels of the locality.

The study area Morigaon district of Assam lies in between 26.12° N to 26.30° N latitude and 91.98° E to 92.28° E Longitude covering a total geographical area of 1704 km², supporting 775874 total population (2001). Out of which 15.4% its population is accounted as Schedule tribe population. *Lalung* peoples are more or less scattered in all the revenue village of the district. As the district lies in the regime of monsoon climate of the sub-tropical belt enjoy average annual rainfall of 1928 mm with a hot and humid summer temperature ranging between 33°C to 24°C and a cool sunny winter with scanty rainfall. The district is extremely flat falling with in imperceptible gradient towards the north-west and essentially a deep alluvial plain of depositional origin except a few patches of high ground and isolated hillocks mostly located in the interfluves represent the worn down spurs of the Meghalaya plateau. The river Brahmaputra forms the northern boundary and leaves the district in the west at 52m above m.s.l. near Hatimuria hill (219m). The district is criss- crossed by a number of comparatively small and meandering river channels- sonai, Mora-sonai, Sondoba and Pakaria flow more or less parallel to Kalong-kapili river (Map No.1) towards north-west in sluggish nature and dotted with series of ox-bow lakes, beels and swamps. The district has 144 numbers of different types of wetland covering 11658 hectares of land area (ARSAC, 1997), where 37 Lake/Pond, 41 Ox-bow Lake/ meander cur-off, 4 seasonal waterlogged, 59 Swamp/marsh, 3Tanks. During summer monsoon period the flood water of the river Brahmaputra filled up all these river channels, marshes and lakes which provide an ideal geo-ecological situation for breeding ground of different species of native fishes. Perhaps considering geo-ecological landscape coupled with the diversity flora and fauna the *Lalungs* of Morigaon district caught fishes in the *beels* using the local growing plant species (Plate no. 1). Following books and journals were consulted (Anonymous, 1994; Bora *et al.*, 1999; Baishya and Majumder, 1980; Borthakur, 1981; Deka *et al.*, 2006; Hajara *et al.*, 1981; Jain, 1989; Jain and Borthakur, 1980).

MATERIAL AND METHODS

The *Lalung* tribe, their fishings activities, plants related to and fishes caught by them are the materials herein. Methodological approach wreath is the periodical field survery, have done for two years (January 2004 to January 2006) among the *Lalung* tribal villegers of Morigaon district. Informations about important useable plants in fishing activities were collected from local *Lalung* people through interview and group discussions. Local name of the plants were collected with the help of experienced local *Lalung* tribe. Voucher specimens were properly identified and compared with standard literatures and records of Botany Departmental herbarium, Gauhati University. Listed plant species are

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Table 1 : Plant names use in Fishing Activities by *Lalung* tribe of Morigaon district in Assam.

Sl. No.	Botanical name	Family name	Local name	Dicota/ Monocota	Habit	Useable part	Uses
1.	*Areca catechu* L.	Arecaceae	Tamul gas	M	Plam	Stem	Use to catch fish in pond, tank and small beel (Hula/Nala).
2.	*Artocarpus heterophyllous* Lamk.	Moraceae	Kathal	D	T	Stem	Wood use in making fishing canoe.
3.	*Bambusa pallid* Munro.	Poaceae	Bijulibah	M	Bamboo	Stem	Use as Fishing rod and Handle of Fishing Javelin.
4.	*Bambusa tulda* Roxb.	Poaceae	Jatibah	M	Bamboo	Stem	Use to make fishing tackles, Fish weir, Fish Garth, Fish creel, Fishing rod, Rowing stick, Handle for Fishing Javelin, use to drop the net.
5.	*Barringtonia acutangula* (L) Gaertn.	Barringtoniaceae	Hijal	D	ST	Bark	Bark boil in water and use to give black colour of Fishing net.
6.	*Boehmeria nivea* Hook. et. Am.	Urticaceae	Riha	M	US	Fiber	Use to knitting of Fishing net.
7.	*Brassica nigra* (L.) Koch.	Brassicaceae	Sariah	D	H	Oil cake	Use as Fish food to motivate the fish.
8.	*Calamus tenuis* L.	Arecaceae	Bet	M	Cane	Cane	Use to knitting and tie in different type of Fishing tackles, Fish garth and Fish creel.
9.	*Corchorus capsularis* L.	Tiliaceae	Marapat	D	H	Fiber	Use to knit Fish weir, tie and different type of rope for different uses.
10.	*Diospyros enbryopteris* Pers.	Ebenaceae	Kandu	D	T	Fruit	Fruits boil in water to give black colour of the Fishing net.
11.	*Diospyros taposia* Ham.	Ebenaceae	Kandu	D	T	Fruit	Fruits boil in water and use to give black colour of the Fishing net.
12.	*Eichhornia crassipes* (Mart.) Solm.	Pontederaceae	Mayteka	M	H	Whole Plant	Use to make Fishing hook-Punga.
13.	*Gmelina arbrea* Roxb.	Verbenaceae	Gamari	D	T	Stem	Fishing boats are prepared from its wood.
14.	*Gossypium barbadense* L.	Malvaceae	Kapah	D	St	Fiber	Use in knitting Fishing Net, String for fishing-Hook.
15.	*Lagerstroemia flos-reginae* Retz.	Lythraceae	Ajar	D	T	Stem	Fishing boats are prepared from its wood.
16.	*Lagerstroemia reginae* Roxb.	Lythraceae	Ajar	D	T	Stem	Wood use to fit out Fishing boat.
17.	*Phragmites karka* Trin-ex steud.	Poaceae	Khagari	M	Grass	Stem	Use as punga of the Fishing net and Fishing hook.
18.	*Polygonum hydropiper* L.	Polygonaceae	Bihlangani	D	Cl	Whole plant	Use as poison to catch fish in hole.
19.	*Psidium guajava* L.	Myrtaceae	Madhuriam	D	T	Leaf	Leaf boil in water and use to give black colour of the fishing net.
20.	Streblus asper Lour.	Moraceae	Sarua	D	ST	Branch	Branch put in pond to give nest for fishes.

N.B.
D = Dicotyledon, M = Monocotyledon, T = Tree, St. Sh. = Straggling shrub, ST = Small tree, Cl = Climber, H = Herbs, US = Under Shurb

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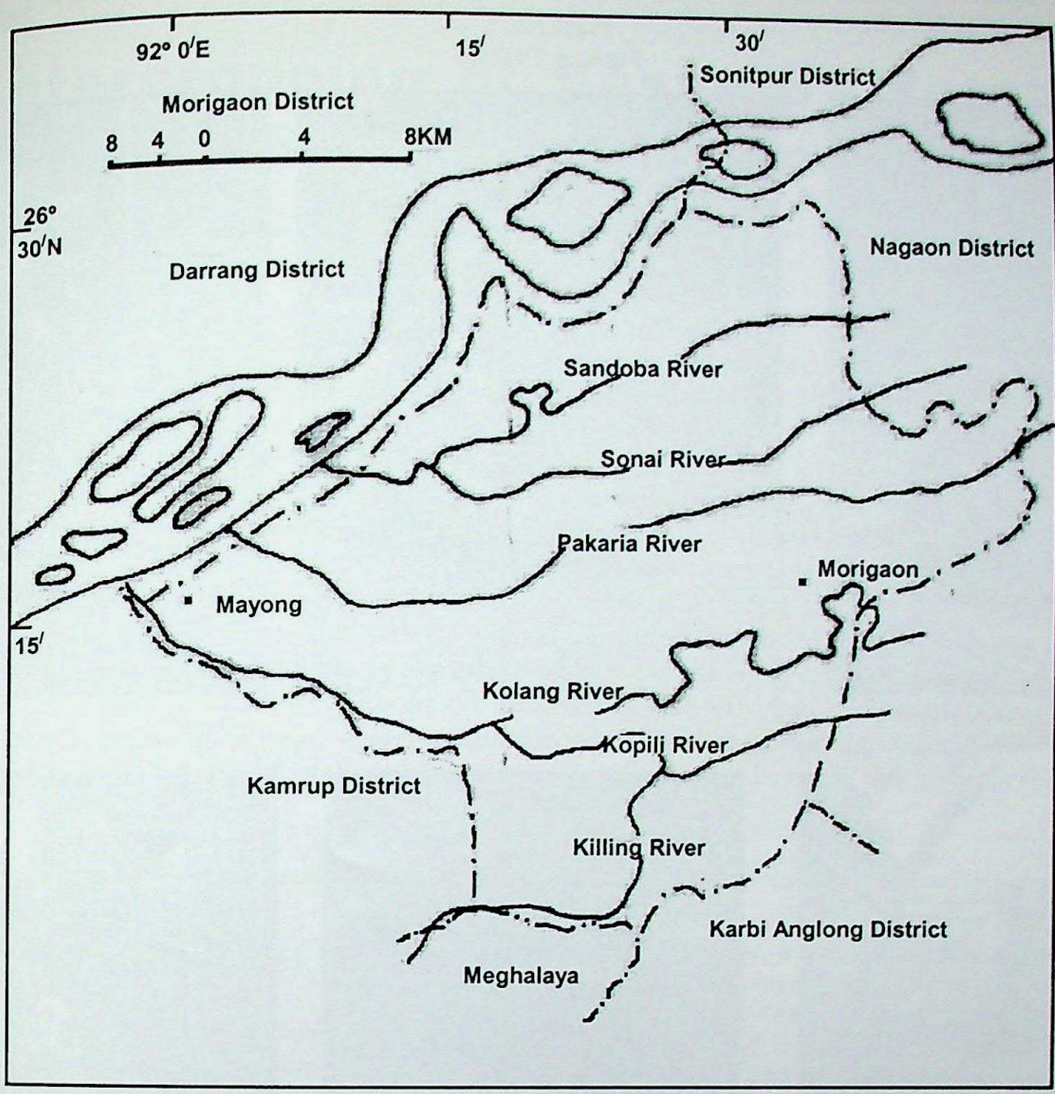

arranged in Table No. 1 as Botanical name, Family name, Local name, Dicotyledon/Monocotyledon, Habit, useable part and uses.

RESULTS AND DISCUSSION

It is observed that *Bambussa tulda* species ranks high in terms of use in fishing activities. The two species *Calamus tenuis* and *Corchorus capsularis* are used in knitting fishing instruments rank second. *Gmelina arbor, Lagerstroemia reginae, Lagerstoemia speciosa* are used in boat making of transpot in fishing, ranks third, *Barringtonia acutanula, Diospyros embroyopteris, Diospyros taposia, Psidium guajava* are used to give black colour in the fishing net.

The tribal people are to some extent maulded by local plants. They are also experienced with the geo-ecological situation of locality and rational use of local natural resource such as flora and funa considering their conservation. They also consider the economic aspects of available natural resources.

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Plate No. 1
Fishing Tackles

Deka, S.J., Ahmed, M. and Deka, S.P. 2006. Survery of Medicinal Plants Used by Tribal (*Lalung*) People of Mayong Area. Morigaon District, Assam, India, Plant Archives **Vol. 6 No. 3**. pp. 667 - 670.

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Intercropping of Kesseru (*Heteropanax fragrans* Seem) with Commercial Crops for Higher Economic Returns

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Kesseru (*Heteropanax fragrans* Seem) has also been regarded as primary host plants of eri silkworm (*Samia ricini* Donovan) which produces eri silk used to prepare quilt, chaddars , scarves and various high valued products. To enhance the profitability of ericulture, it is important to introduce high valued crops as components of eri food plant based intercropping besides, extending their cultivation to non-traditional areas. Hence, three kesseru based intercropping systems were evaluated considering ginger, turmeric and colocasia as components in Research Extension Centre, Mongaldoi under Central Muga Eri Research and Training Institute, Lahdoigarh, Jorhat, Assam. It was observed that the different cropping systems had no adverse effects on the main crop, Kesseru. The growth parameters and leaf yield of Kesseru plants grown under intercropping were comparable to Kesseru plants grown under sole cropping. Among the three systems, Kesseru + Colocasia was found as the most suitable cropping system as it had the highest return per rupee invested.

Keywords : Intercropping systems, Leaf yield, Growth parameters, Return per rupee invested.

INTRODUCTION

Eri silk is used to prepare various readymade garments, bed-spreads, carpets, cushion covers, shirts, cocoon handicrafts and other fashion accessories which have high market value. The producer of eri silk, *Samia ricini* Donovan (Family: Saturniidae) is found in Assam, Meghalaya, Manipur, Arunachal Pradesh, Nagaland, Mizoram, Orissa, West Bengal, Bihar and Andhra Pradesh. Eri culture is practiced as a cultural tradition by different tribes and sub-tribes of the Northeastern region both in plains as well as hills where the rearer is the producer, the processor and also the buyer. The main purpose of eri rearing in the region is mainly for its pupae which is rich in vitamins and other nutrients and form a delicious item of food for a large section of population. The other purposes are preparation of quilt, chaddars and scarves, generally preferred in cooler and hilly areas which help the farmers to cut down considerable expenditure on woolen clothes. Moreover, Eri Industry provide jobs/income resources for fast increasing populations engaged in farming as well as to children, unemployed youth and women belonging to rural areas of the region.

Eri silkworm (*Samia ricini* Donovan) feeds on leaves of varieties of floral species, of which castor (*Ricinus communis* L.) is the most preferred one because castor leaf is most palatable, eri cocoons obtained in castor are of bigger in size, cocoons contain more silk and produces quality fibres.

However, a successful harvest of cocoon on a large scale depends on selection of suitable host tree- permanent in nature like Kesseru (*Heteropanax fragrans* Seem) of Araliaceae and Barkesseru (*Ailanthus excelsa* Roxb.) of Simaroubaceae family . The wild eri worm (*Samia cynthia*) is found naturally feeding on Borkesseru and Kesseru leaves. Raising early stages worm, upto third stage on castor leaf, followed by kesseru at the last stage would ensure a larger harvest with least labour avoiding various hazards. Hence, kesseru has also been regarded as primary host plants of eri silkworm.

For cultivation of kesseru, 2m x 2m and 3m x 3m spacings are followed. August to September is the ideal season for kesseru cultivation. It takes at least 2 years for establishment and after establishment; pruning is done at a height of 1.75 m to keep the plants at a manageable height. Leaves are harvested 2 -3 times in a year for silkworm rearing. In 1 ha systematic kesseru plantation, 37.5 % area remain unutilized which can be utilized by planting various intercrops for better utilization of land and other resources. It is observed that the active root zone of kesseru is confined to 60 per cent of the available land area and the remaining area could be profitably exploited for raising subsidiary crops. Further, during the three years gestation period of kesseru growth, sufficient amount of sunlight is transmitted through canopy of kesseru and falling on the ground, which is suitable for growing any annual/biennial crop, because at that time, intercrops have minimal competition with kesseru tree for ecological factors. The crown structure of full-grown kesseru allow only part of the incident solar

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radiation to pass through the canopy and this diffused sunlight may facilitate growing of a number of shade loving crops in the interspaces. Therefore, the unutilized land and the diffused sunlight in mature kesseru plantations can effectively be utilized by judicious selection of partial shade loving intercrops.

Further, eri culture is a labour intensive process and around 50-60% of the total cost of production being spent for silkworm rearing activities. As a result, farmers are not getting better returns which is definitely influencing their choice for the crop. So, to enhance the profitability of eri culture, we can introduce high valued crops as components of kesseru based cropping system.

Various workers suggested various crops for intercropping to increase farm income. Nelliat *et al.* (1974) reported intercropping of ginger and turmeric in coconut and arecanut plantation. Higher yields of ginger and turmeric as intercrops with coconut compared to open space was reported by Jayachandran *et al.* (1991) which indicates that ginger and turmeric are shade loving crops. This finding is in conformity with the findings of Nizam and Jayachandran (1977) who reported production of higher quality rhizomes in ginger when it was grown under coconut shade. Further, Jayachandran *et al* (1998) reported 32% increase in rhizome yield of ginger under natural shade in the coconut plantation. Again, Ancy and Jayachandran (1998) reported that high yield of ginger under shaded situation may be due to higher nutrient uptake. As ginger and turmeric are important rhizomatous crops, used in culinary purposes and indispensable in the manufacturing of number of food products as well as industrial products, ginger and turmeric can be grown as intercrops to increase the production also. Colocasia is another shade loving tuber crop grown as intercrop in coconut plantation. Later on, Kumar *et al.* (2001) reported ginger as the most suitable intercrop with *Ailanthus* spp. However, intercrops should be selected according to the tolerance of crops to various diseases, soil type, rainfall pattern/irrigation facilities, climatic conditions, availability of marketing/processing facility and labour availability.

Keeping above as background, a study was conducted on intercropping of these three commercially valued shade loving crops with the following objectives-

- To determine the effects of different cropping systems on the growth and leaf yield of kesseru.
- To determine the benefit cost ratio of each cropping system and
- To determine the most suitable kesseru based cropping system.

METHODOLOGY

The study was conducted for two consecutive years during 2006-07 and 2007-08. The experimental site was sandy loam in texture and very acidic (Soil pH 4.5) in reaction.

Existing 3 years old kesseru plantation under recommended 2m x 2m spacing was selected as main crop for the study and maintained through recommended package of practices. Three commercially valued crops *viz.*, Ginger (*Zingiber officinale* Rosc.), Turmeric (*Curcuma longa* L.) and Colocasia (*Colocasia esculenta* (L.) Schott) were grown as intercrop under kesseru plantation in four cropping combinations namely T1 : kesseru + Ginger; T2: kesseru + Turmeric, T3: kesseru + Colocasia and T4: kesseru sole cropping (control). The variety of Ginger, Turmeric and Colocasia selected for the study were Bhola, Tall clone and Ahinia kachu respectively.

The experiment was laid out in RBD with 4 replications. Size of each experimental plot was 120 sq.m. (12m x 10m). The number of kesseru plants in each plot was 30. The intercrops were sown as per treatment in the second week of March every year in the effective inter spaces in between two rows of kesseru plantation following recommended spacing of each intercrop, *viz.*, ginger (25 cm x 20 cm), turmeric (45 cm x 25 cm) and colocasia (60 cm x 45 cm). The area under intercrop in each plot was 40 sq.m. The population of ginger, turmeric and colocasia per plot was 800, 352 and 128 respectively. A fertilizer dose of 125:75:25 Kg NPK/ha only was applied to kesseru plantation in T4 where as in other treatments, in addition of 125:75:25 Kg NPK/ha to kesseru plantation, recommended fertilizer dose was applied to different intercrops. Eri silkworm rearing was conducted in three seasons *viz.*, May-June, Oct.-Nov. and Feb.-March and cocoons were harvested. Economics of the treatments was worked out on the basis of prevailing market prices of inputs and outputs. Data on growth parameters of kesseru, yield of kesseru and intercrops, rearing performance of eri silkworm on kesseru plants, production cost and income were gathered. To know the significance of differences among the treatments critical difference was calculated. A market price of Rs.7/-, Rs. 4/- and Rs. 7/- per Kg of ginger, turmeric and colocasia was used in calculation of economics.

RESULTS AND DISCUSSION

A. Growth parameters and Leaf yield of kesseru : Plant height of kesseru in kesseru + ginger cropping system decreased significantly than those of sole cropping of kesseru and other two intercropping systems. However, plant height in association with turmeric and colocasia did not differ significantly than that of sole cropping of kesseru. Crown width in association with turmeric did not differ significantly than that of sole cropping of kesseru but ginger, when grown as intercrop improved crown width significantly and colocasia as intercrop reduced crown width significantly. On the other hand, crown length in association with intercrops did not differ significantly than that of sole cropping of kesseru.

A reduction in green leaf yield of kesseru occurred due to intercropping , however, there was no significant difference in leaf yield between sole cropping and intercropping systems of kesseru. This result agrees with the findings of Rabe (1984) in which intercropping of commercial cash crops with abaca (*Musa texilis*) did not significantly affect suckering ability of the main crop.

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Table 1 : Effect of intercropping systems on Leaf yield and growth parameters of kesseru .

Cropping system	Green Leaf yield (t/ha/yr)	Plant height (m)	Crown width/pl. (m)	Crown length/pl. (m)
Kesseru + Ginger	25.6	3.44*	3.40*	2.61
Kesseru + Turmeric	25.8	3.77	3.06	2.58
Kesseru + Colocasia	26.2	3.75	2.73*	2.67
Kesseru sole cropping	26.4	3.71	3.05	2.77
SEm ± (0.05)	**0.39**	**0.48**	**0.18**	**0.08**
CD at 5%	**NS**	**0.17**	**0.06**	**NS**

Table 2 : Effects of cropping systems on growth and yield of intercrops.

Cropping system	Plant height (cm)			Leaves/plant (No.)			Yield (t/ha)		
	Ginger	Turmeric	Colocasia	Ginger	Turmeric	Colocasia	Ginger	Turmeric	Colocasia
As sole crop	60.0	84.5	105.5	28.5	7.60	17.5	10.53	14.56	12.88
As intercrop	54.0	87.5	127.5	28.0	7.20	9.0	4.23	5.40	4.67
SEm ± (0.05)	**2.12**	**2.83**	**4.24**	**4.60**	**0.28**	**4.60**	**0.16**	**0.22**	**0.23**
CD at 5%	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Table 3 : Effects of intercropping systems on rearing performance of eri silkworm.

Cropping system	Larval weight (g)	ERR(%)	Cocoon weight (g)	Shell weight (g)	SR (%)
Kesseru + Ginger	5.25	68.73	2.86	0.29	10.25
Kesseru + Turmeric	5.61	69.06	3.09	0.33	10.58
Kesseru + Colocasia	5.61	70.24	3.08	0.28	9.21
Kesseru sole cropping	5.15	65.92	3.04	0.28	9.18
SEm ± (0.05)	**0.18**	**0.38**	**0.12**	**0.19**	**0.38**
CD at 5%	**NS**	**1.33**	**NS**	**NS**	**NS**

Table 4 : Effects of intercropping systems on rearing performance of eri silkworm.

Cropping system	Eri cut cocoon Yield (Kg)	Eri Pupa yield (Kg)
Kesseru + Ginger	128	852
Kesseru + Turmeric	129	860
Kesseru + Colocasia	131	872
Kesseru sole cropping	132	880

Table 5 : Income and Benefit cost ratio (BCR) in different cropping systems.

Cropping system	Cost of production (Rs./ha/yr)	Annual Gross income (Rs./ha)	Annual net income (Rs./ha)	Increase in income over sole cropping (Rs./ha/yr)	Per cent increase over sole cropping	BCR
Kesseru + Ginger	84,695	95,640	10,945	4,045	58.62	1.13
Kesseru + Turmeric	76,225	88,250	12,025	5,125	74.28	1.16
Kesseru + Colocasia	79,270	100,270	21,000	14,100	204.3	1.26
Kesseru sole cropping	61,300	68,200	6,900	-	-	1.11

B. Growth and yield of intercrops : All the intercrops grew well in the effective interspaces (3333sq.m/ha) of kesseru plantation. Ginger, turmeric and colocasia yielded 4.23 t rhizome /yr, 5.40 t rhizome /yr and 4.67 t corms/yr. respectively when grown as intercrops in the effective interspaces of one hectare kesseru plantation against 10.53, 14.56 and 12.88 t yield/ha/yr in sole cropping systems of respective intercrops. The reduction in yield of intercrops is attributed to decrease in proportionate area of crops in intercropping.

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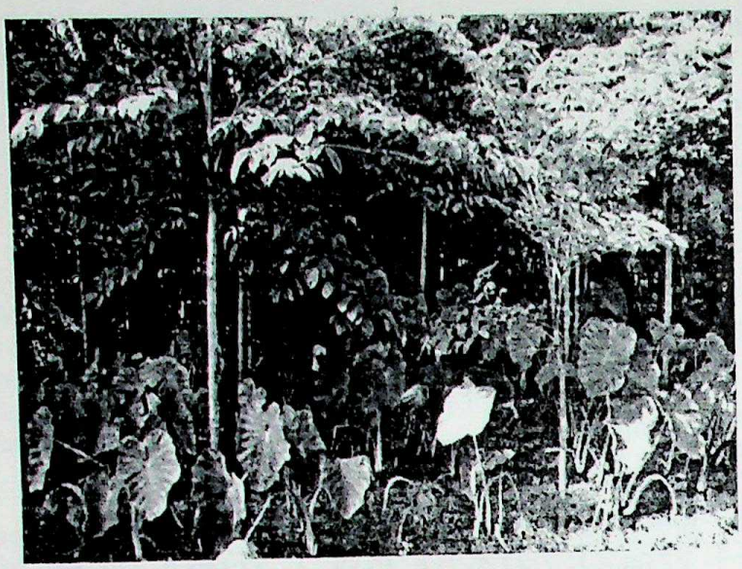
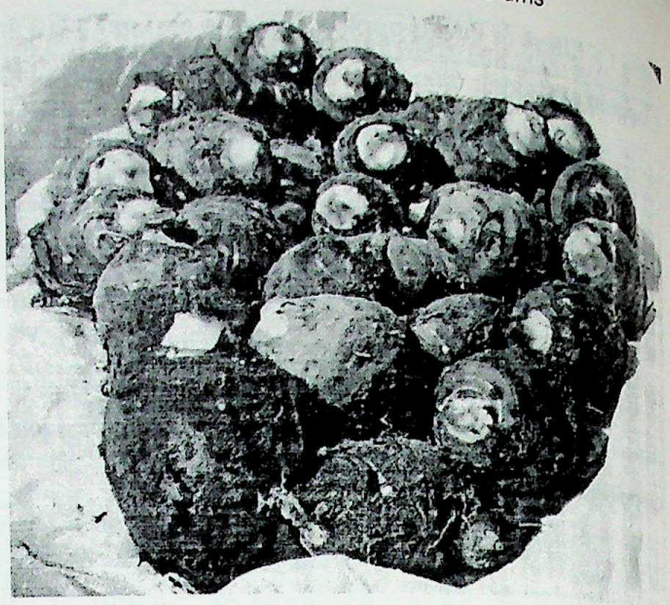
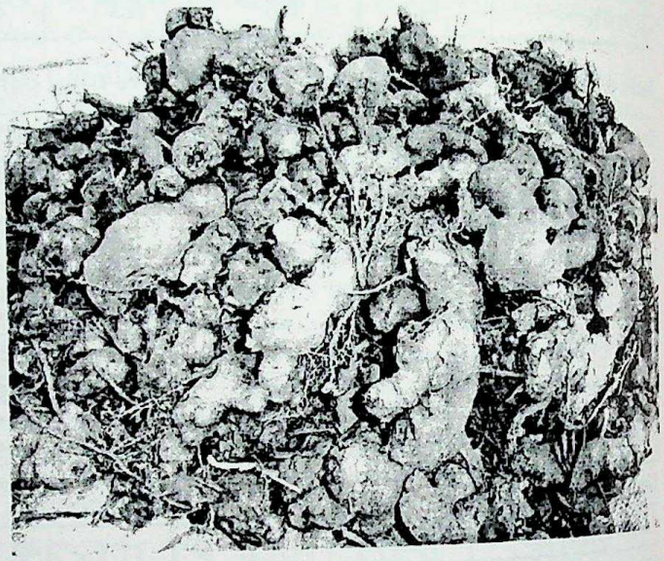
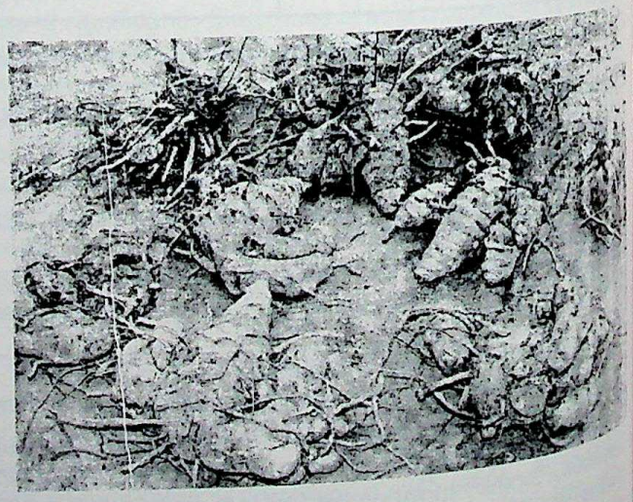




Colocasia intercropped with kesseru



Ginger intercropped with kesseru



Turmeric intercropped with kesseru

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C. Rearing performance of eri silkworm : Bioassay on various economic characters *viz.*, larval weight, cocoon weight, shell weight and silk ratio registered no significant difference between the intercropped and sole cropped plots of kesseru which indicates no adverse effect of intercropping on palatability and nutrient status of kesseru leaves. Rather, ERR increased significantly in intercropping systems than kesseru sole system which indicates probable beneficial effect of intercropping on palatability and nutrient status of kesseru leaves.

D. Production of eri cut cocoon and pupa : Kesseru sole cropping produced 26.4 t green leaves /ha/yr which in turn, through eri culture produced 1.32 q eri cut cocoons and 8.8 q eri pupa. Among the intercropping systems, the maximum production of cut cocoons (1.31 q) and pupa (8.72 q) was obtained in kesseru+ colocasia cultivation.

E. Economics : Kesseru sole cropping resulted a net return of Rs. 6900/ha/yr. On the other hand, intercropping enhanced net returns over sole cropping of kesseru. Kesseru + ginger cultivation enhanced net return by 58 % , Kesseru+ turmeric cultivation by 74% and Kesseru + Colocasia cultivation enhanced by 204% . Highest additional net returns of Rs. 14,100/ha accrued from Kesseru + Colocasia. Benefit cost ratio (BCR) was also the highest (1.26) for Kesseru + Colocasia system.

CONCLUSION

The study revealed that intercrops grew well in the interspaces of kesseru plantation. Proper manuring and adoption of scientific intercrop management practices improved growth and development of main crop also. Further, shade of kesseru plants enhanced the growth and development of intercrops over sole cropping. From BCR point of view, all the intercropping systems were profitable than sole cropping, however, Kesseru + Colocasia. system accured highest net returns (Rs. 21,000/ha) and highest Benefit cost ratio (1.26) which indicates more profitability of the system than other systems.

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Present Status of Mulberry Varietal Improvement in India

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Mulberry (*Morus* spp.) of the family moraceae, the promising food plant of silkworm (*Bombyx mori* L.) - a perennial, deep rooted, widely adoptable, fast growing with indeterminate type of terminal plant growth, high biomass producing protein rich foliage plant - is basically a tree species. However, for purpose of intensified cultivation it is grown as bushes of different sizes varying up to small trees depending on the suitability of the situation under cultivation.

INTRODUCTION

Improvement in the production of silk involves first and foremost improvement in yielding ability of mulberry, the host plant of silkworm, *Bombyx mori*. L. Improvement in a crop variety indicates mostly the genetically improvement which may be considered as permanent one. Allard (1960) rightly opined that the contribution to human welfare made by superior varieties is the most satisfying of all the methods of increasing production.

The improvement in productivity and quality of mulberry could be achieved by developing a superior variety and by creating a suitable environment that favors the expression of full potentiality of the genotype. No matter how excellent the genotypes are, maximum production cannot be achieved unless good management practices are followed along with the improved genotypes.

In the beginning (early phase) exploration and selection was made from local land races. Introduction was followed and varieties such as Ber-S-1 (CSRTI, Berhampore) and Goshoerami (Jammu & Kashmir) were introduced.

MATERIAL AND METHODS

Objectives of Mulberry Breeding

1. Improving yield and quality in productive areas to compete with other commercial crops and to achieve vertical growth.
2. Varieties tolerant to abiotic stress like soil moisture stress, alkalinity, salinity and acidic soils.
3. Varieties resistant to root and foliar diseases and pests of mulberry.
4. Varieties suitable to grow as intercrop, mechanized cultivation, exclusive chawki garden, under low input conditions and to raise as small trees etc.,
5. Development of varieties for specific agro-climatic zones.
6. Development of fertilizer responsive varieties.
7. Development of photo and thermo insensitive varieties.

Problems Associated with Mulberry Breeding

Viewing the limited success in developing new mulberry varieties, it is apparent that there are some inherent problems, which hinder the development process. These problems are listed below :

1. As mulberry is tree in nature, the generation time is quite long and such long generation time does not permit to use different breeding processes that are generally followed for annual cross pollinated crops.
2. Poor relationship of the important characters in juvenile stage with that of the yield in adult stage makes the prediction difficult at the young age.
3. Mulberry is highly cross-pollinated and heterozygous crop and no pure line is still available.
4. Non-availability of pure line limits our knowledge with respect to the inheritance pattern of important economic characters.
5. Tissue culture technique failed to provide sufficient useful information in mulberry. Apart from the success in developing protocol for micro-propagation, attempt to develop haploid and double haploid plants were mostly unsuccessful except for a sporadic report published by the Chinese scientists. In so many crop plants protoplast culture provided an opportunity to develop superior genotypes by somatic hybridization, but in mulberry fusion and regeneration of protoplast have not been achieved very successfully.

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Conditions Favouring Mulberry Breeding

1. Easy fixation of heterosis - Mulberry is easily propagated through stem cuttings. Once a superior genotype is identified, at any stage of developmental process can be preserved or fixed through cuttings and grafting.

2. Due to the cross-pollination and heterozygous nature of the parents, the progeny immediately exhibits variation on crossing. Such variability promises the success of conventional breeding.

3. Mulberry is a unique plant, which show high plasticity with differential response under different conditions. Such response provides protection in different environments (Gray and Gray, 1987). The ecological and geographical distribution of mulberry varies from intensive cultivation as food for silkworm to the natural occurrence in forests and urban areas. The phenotypic plasticity in mulberry contributed to its ability to grow and survive under disruptive environmental condition (Gray, 1990). This opens up the opportunity to develop varieties suitable to new agricultural areas.

RESULTS AND DISCUSSION

Studies conducted to aid breeding programme in mulberry :

Evaluation and characterization of germplasm : To make the breeding programme more directional, lots of basic studies were conducted in India and abroad, but unfortunately those basic information were not used in a systematic manner in developing a high yielding mulberry variety. It is quite well known that collection and evaluation of germplasm is a basic requirement for initiation of breeding programme. Machii *et al.*, (1997) have published an exhaustive guideline for characterization and evaluation of mulberry genetic resources. Under the model proposed by them, the characters were broadly classified under:

Table 1 : Area wise problems and requirements.

Areas	Problems	Requirements
Hills and plains of north-eastern regions	(a) Acid soil (b) More acceptability of other cash crops like ginger, etc., (c) Higher incidence of mildew.	Tolerant to acidity. Suitable plant type for intercropping. Tolerant to diseases.
Eastern region	(a) Medium yield. (b) Low yielding during winter season. (c) Higher incidence of leaf spot diseases in rainy season and mildew in winter. (d) Soil salinity in coastal areas.	High yield. Stability of yield in winter season. Tolerant to diseases. Tolerant to salinity.
Temperature (Northern hill)	(a) Soil acidity (b) Low rooting (c) Cold (d) High dormancy	Tolerant to soil acidity. Superior genotypes with high rooting. Tolerant to frost and cold. Less dormancy.
Southern region (Irrigated)	(a) Medium yield. (b) Competition with sugar cane and other horticultural crop. (c) High incidence of leaf spot and leaf rust. (d) Nematode infestation.	High yield. Varieties favoring mechanization. Disease tolerant. Resistant to nematode.
Southern (Semi-arid rain fed region) Coastal	(a) Scarcity of water. (b) High atmospheric temperature, low humidity and high wind velocity. (c) Erratic rainfall. (d) Poor annual rainfall with uneven distribution. (e) Poor yield. (f) Soil alkalinity.	Tolerant to water stress. Drought tolerant. Comparatively high yield. Tolerant to alkalinity.
Salinity.	(a) Saline soil. (b) Saline irrigation water.	Tolerant to salinity.

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Table 2 : Mulberry varieties presently under cultivation in India.

Variety	Region	Developed at	Breeding methods used	Leaf yield in resp.areas (MT/ha/yr)
Kanva-2	South India Irrigated	CSRTI, Mysore	Selection from natural variability	30-35
S-36	- do -	- do -	Developed through EMS treatment of Berhampore Local.	40-45
S-54	- do -	- do -	- do -	45-48
Victory-1	- do -	- do -	Hybrid from S30 x Ber C776	65-70
DD	- do -	KSSRDI, Thalaghattapura	Clonal selection.	40
S-13	South India Rainfed	CSRTI, Mysore	Selection from polycross (mixed pollen) progeny.	12-15
S-34	- do -	- do -	Selection from polycross (mixed pollen) progeny.	12-15
MR-2	- do -	- do -	Selection from open pollinated hybrids.	35
S-1	Eastern and N.E. India Irrigated	CSRTI, Berhampore	Introduction from (Mandalaya) Burma.	30-35
S799	- do -	- do -	Selection from open pollinated hybrids.	35-40
S1635	- do -	- do -	Triploid selection.	40-45
S146	N.India and Foothills of J & K Irrigated	- do -	Selection from unknown source.	30-35
Tr-10	Hills of Eastern India	- do -	Triploid of Ber.S1.	20-25
BC_2-59	- do -	- do -	*M.indica* (Local) crossed with Kosen and back cross twice with Kosen.	18-20
Goshoerami	Temperate	CSRTI, Pampore	Introduction from Japan.	15-20
Chak Majra	Sub-temperate	RSRS, Jammu	Selection from natural variability.	25-30
China White	Temperate	CSRTI, Pampore	Clonal selection.	15-20
Anantha	South India Irrigated	RSRS Anantapur	Clonal selection from RFS-135	55-60
RG-120	Eastern India	CSRTI, Berhampore	Cross between CF_1-10 x C763	55-56

1. Essential and optional primary characters, which include mostly morphological parameters characterizing stem, leaf, petiole, flowers and seeds.
2. Essential and optional secondary characters which include sprouting and rooting, tolerance to varied climate and diseases etc.,
3. Essential and optional tertiary characters, which include mostly the quantitative characters such as, shoot weight, leaf weight and nutritional status, mostly the nitrogen content of mulberry leaf.

In order to find out the important parameters contributing to the selection of desirable genotypes in mulberry, association of important yield contributing parameters were studied and it was found that total length of all shoots, total weight of all branches and 100 leaf weight exhibited high direct effects and also showed high significant correlations with leaf yield (Rahman *et al.*, 1994). The study on indirect selection indicated the similar picture (Sarkar *et al.*, 1992). Further analysis of data indicated that these above mentioned three characters had high correlation with leaf yield both at genotypic and environmental level. Further, for the corresponding character pair the correlations both at genotypic and environmental level did not differ significantly indicating the existence of physiological basis (Sarkar *et al.*, 1997).

The above mentioned selection parameters were determined after conducting the tests under irrigated condition.

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Table 3 : Parameters considered for screening and selection of mulberry genotypes.

Selection period and observation norms	Parameters
1. First selection/preliminary screening.	
(a) Early observations	Leaf lobation, presence of lateral shoot, leaf colour, leaf surface, appearance, petiole length and disease resistance.
(b) Late observations	Date of sprouting, total shoot length, average shoot length, length of longest shoot, internodal distance, joso yield, weight of shoot, number of leaves/shoot, leaf weight.
2. Second selection/final yield trial	Same parameters as described in (b) of item No.1; chemical analysis of leaves and acceptability to silkworm through feeding trial.

Experiment was also conducted to identify the selection parameters under water stress condition (Mallikarjuna, 1997). Interestingly, the results were not much different with that obtained under irrigated condition. A large number of parameters were considered covering morphological (4), root (3), anatomical (9), physiological (9) and biochemical (7) parameters. The study indicated that total length of shoots/ plant, weight of leaf per plant, number of shoots/plant and leaf area/plant were among the morphological and leaf surface wax and proline content were the most crucial parameters contributing to the leaf yield during water stress period. The stepwise regression analysis indicated the necessity of using above six parameters jointly to keep the prediction level at 95.5%. It is further interesting to note that neither any of the root parameters nor any of the physiological parameters contributed towards yield under water stress condition. Out of the nine characters studied under anatomical parameters only number of stomata had some contribution towards yield.

Breeding for region specific mulberry varieties : In developing region specific mulberry varieties, direct breeding at the respective regional research station was suggested. But, due to the lack of facilities and expert staffs the programme did not yield any results. Indirect breeding, *i.e.,* testing the promising genotypes developed at the main institute offers the best solution and was followed by Regional Sericultural Research Stations.

Other breeding methods followed : Apart from the conventional breeding technique used for development of high yielding mulberry, polyploid breeding and mutation breeding were also attempted earlier in India, China and Japan and in the erstwhile USSR. Out of these two techniques, polyploidy breeding showed more promises and a number of varieties were developed in India, Japan and the USSR (Abdullaev *et al.*, 1970, Das *et al.*, 1970 and Dzhafarov *et al.*, 1973). In India, triploid varieties like Tr 8 and Tr 10 have been recommended in the hill areas of Eastern India. Triploid mulberry varieties are generally superior in feeding value in comparison to their diploid counterparts (Das and Prasad, 1974, Dzhafarov and Gadzheiev, 1972). Moreover, triploids have been reported to be tolerant to problematic soil.

Mutation breeding : It was earlier thought that mulberry might be the suitable plant to respond to the mutational research. Lots of works were conducted in Japan at the Institute of Radiation Breeding, Ibaraki, Japan, since 1957 (Katagiri, 1970, Nakajima, 1971) but no useful mutant was recommended in the field so far. Hence, mutation breeding in mulberry is considered as low priority research area.

Future strategies for mulberry improvement : Future strategies towards mulberry improvement may be decided depending on the future need. The earlier strategies were to develop mulberry varieties suitable to irrigated and rain fed conditions only. But at the present age, the objectives have become more specific and hence more attentions are required to tackle the problems.

Future prospects through biotechnological researches : In the coming years, it is presumed that biotechnological researches will progress to an extent that the genes for resistance to different stress factors (both biotic and abiotic) will be available and could be incorporated at any desired genetic background. Lal and Coworkers (2007) studied the over expression of HAV1 gene from barley and showed the tolerance to salinity and water stress in transgeneic mulberry. Kamble (2008) narrated the Seri biotechnological research in India and given the details of micro propagation, somatic hybridization and genetic engineering in mulberry. A novel system of the combination of conventional and biotechnological approach is expected to increase the yield through genetic up gradation of the cultivars.

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Studies on Diversity of *Costus speciosus* (Koenig.) Sm. (Zingiberaceae) in Assam

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An investigation on the diversity of *Costus speciosus* (Koenig.) Sm. was conducted in Assam. Based on general superficial morphology three morphological variants were recognized and general morphological characters specially stem size and height, leaf-morphology and leaf-epidermal features were studied by following standard methods. The general morphology as well as leaf-epidermal features analysis revealed there is an inter-varietal variation in *Costus speciosus* (Koenig.) Sm. in Assam.

Keywords : *Costus speciosus*, Zingiberaceae.

INTRODUCTION

Assam, a state in the North-Eastern region of India, with a geographical area of 78, 438 sq. km.(88° 25 - 96° 0 E latitude and 24° 5 - 28° 0 N longitudes) with hills and plains, is known for its rich flora and diverse forests and vegetations due to its unique topography, climate and altitude patterns. Average rainfall is 2000 mm with seasonal variation (Sharma 2004). The mean maximum temperature is 37°C in summer and 12°C in winter. The forests are generally evergreen, semi-evergreen to deciduous type.

The foliar epidermal characters play an important role in the field of taxonomic and phylogenetic studies of taxa (Stace, 1965). Beside the gross morphological characters the leaf epidermal characters variation is an important character which can successfully be used to determine the inter-varieties relationship of different plants. The use of quantitative microscopy of leaves in distinguishing taxa is found useful in the members of Solanaceae (Pandey, 1982). Rao and Raju (1985) made a survey on the foliar trichome of the family Euphorbiaceae.

Costus speciosus (Koenig.) Sm. belongs to family Zingiberaceae. It is known as 'Jamlakhuti' locally, 'Keukand' in Hindi and 'Kustha' in Sanskrit (Mandal *et al.,* 2007). It is a succulent perennial herb, growing up to 3.5 m high and having an erect stem. The plant possesses horizontal rhizomatous rootstock. It has an extensive distribution in India and is more common in Bengal, North Eastern Region and humid tropics of South India. It thrives well on moist soil and shady localities under mixed deciduous forests. In Assam this plant is found in wild as well as in semi-cultivated state. The plant propagates vegetative means through rhizomes or seeds dispersed by birds. Rhizome is the plant part used as medicine. In Ayureveda the rhizomes were ascribed to be bitter, astringent, cooling, purgative, anti-helminthic, expectorant and tonic (Mandal *et al.,* 2007). Ethno-medicinally in Assam the plant is used in curing aliments like burning sensation of urinary tract, constipation, fever, inflammation, worm infection and disease like asthma, bronchitis and anemia (Borthakur & Goswami, 1995), The plant has attained importance recently as a source of disogenin, a precursor in synthesis of steroidal hormones from the rhizome (Sarin, *et al.*, 1974).

Costus speciosus (Koenig.) Sm. of this region generally shows variation in general morphology. The present study was conducted to determine the diversity of the species in this region.

METHODOLOGY

Altogether 20 samples of *Costus speciosus* (Koenig.) Sm. were collected from different agro-climatic regions of Assam. The samples were grown under same environmental conditions. Herbarium sheets were made for identification. For authentic identification local flora and monographs have been consulted.

On the basis of stem characters and leaf morphology samples were classified into three groups as Variant-I, Variant-II and Variant-III.

For foliar micro-morphological characters fresh leaves of all three variants were fixed in 5% formaldehyde solution. Both the epidermal strips were obtained by peeling and scraping respectively. The strips were cleaned in 5% sodium hypochlorite (NaOCl) for 5 minutes, washed in water, stained in 1% safranine for 1 minute and mounted in glycerine. The slides were examined under compound microscope and micro-photographs of the epidermis were taken at (10 x 40) X magnification. The quantitative data were based on the average

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of 10 readings. Descriptive statistics of mean and standard deviation was calculated for all the variables. For the terminology used in this study was followed as adopted by Metcalfe and Chalk (1950).

RESULTS

On the basis length and diameter of stem the samples were classified into following three groups (Photograph - A):
Variant-I: Stem long, white in colour and diameter thick,
Variant-II: Stem long, red in colour and diameter thin,
Variant-III: Stem short, white in colour and diameter thick,

DISCUSSION AND CONCLUSION

The general morphology and leaf micro-morphology of the variants investigated are summarized in Table No. 1 and Table No. 2 respectively. In case of stem length, the variants have shown significant variation (3.5 m, 3 m and 2 m for VR-I, VR-II and VR-III respectively). Likewise, for stem diameter, Variant-II is thin (7 cm), Variant-I and Variant-II are thick (10 cm). In case of leaves, the Variant-I and Variant-III have shown same general structure; but, in case of Variant-II leaves are linear.

Type of stomata is paratetracytic and epidermal cells are polygonal for all the variants investigated. It is observed that the epidermal cells architecture and trichome complex of the variants have shown no significant variation except slight difference in size of epidermal cells.

The stomatal size, stomatal frequency and stomatal index showed significant variation among the variation. The stomatal size observed in case of variant-I: 2.7 ± .25 x 1.5 ± .22, Variant-II: 2.1 ± .28 x 1.1 ± .25, and Variant-III: 2.7 ± .25 x 1.5 ± .15. Stomatal frequency observed on the lower surface of Variant-I : 6 ± 1.3, Variant-II : 8 ± 1.8 and Variant-III : 6 ± 1.5. Likewise, stomatal index observed on lower surface of Variant-I : 11.35 ± 1.5, Variant-II : 6.80 ± .89, and Variant-III : 15.70 ± 1.3; but on the upper surface all the variants showed more or less same stomatal index.

In the present study the three variants are easily distinguishable based on gross morphology characters. That variation in gross morphology serves diagnostic purposes among angiosperm groups is well documented (Davis and Heywood, 1963; Metcalfe and Chalk, 1979). However, it is equally significant to delimit taxa with micro morphological characters in addition (Stace, 1980). stomatal characters (viz. stomatal type, stomatal index, stomatal size, etc.) come in handy in delimiting taxa at the infraspecific level (Obute and Omotayo, 1999).

The general morphology and leaf epidermal features analysis revealed inter-varietal variation in one hand and correlation on the other. In conclusion, these bring out the fact that a genetic variability exists in the natural population of *Costus speciosus* (Koenig.) Sm. In this region. Characters like stomatal size, stomatal frequency and stomatal index can be used to separate the variants in addition to gross morphology. Further studies like isoenzyme technique and karyotype analysis can be conducted to unravel the genetic diversity existing in the plant.

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(A) Photograph of *Costus speciosus* (Koenig.) Sm., three varieties: VR-I, VR-II & VR-III

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Table 1 : Summary of general morphology in the Variants of *Costus speciosus* (Koenig.) Sm. studied.

Characters	Variant-I	Variant-II	Variant-III
Stem:			
Length(average):	3.5 m	3 m	2 m
Diameter(average):	10 cm	7 cm	10 cm
Colour:	White	Red	White
Leaf:			
Shape:	Lanceolate	Lanceolate	Lanceolate
Size (length x breath):	41 x 15.8 cm	29 x 7 cm	34 x 11 cm

Table 2 : Summary of leaf epidermal morphology in the Variants of *Costus speciosus* (Koenig.) Sm. studied.

Characters	Variant-I	Variant-II	Variant-III
1. Epidermal cells structure:			
Shape on upper surface:	Polygonal	Polygonal	Polygonal
Shape on lower surface:	Polygonal	Polygonal	Polygonal
2. Stomata:			
Stomatal size: (length ± S.D. x breath ± S.D) µm	2.7 ± .25 x 1.5 ± .22,	2.1 ± .28 x 1.1 ± .25,	2.7 ± .25 x 1.5 ± .15
Stomatal frequency: Per microscopic Field under (10 x 10)X magnification: (Mean ± S.D.)			
Upper surface:	1 ± .02, (EC=35)	2 ± .05, (EC=67)	1 ± .03, (EC=30)
Lower surface:	6 ± 1.3, (EC=40)	8 ± 1.8, (EC=68)	6 ± 1.5, (EC=32)
Stomatal type:	Paratetracytic,	Paratetracytic,	Paratetracytic,
Stomatal Index (S.I.) ± S.D:			
Upper surface:	2.70 ± 1.23,	2.80 ± .25,	7.60 ± 1.15,
Lower surface:	11.35 ± 1.5,	6.80 ± .89,	15.70 ± 1.3
3. Trichomes:	Multi-cellular, simple, consist of single row of cells.	Multi-cellular, simple, consist of single row of cells.	Multi-cellular simple, consist of single row of cells.

NB: S.D. =Standard Deviation, EC = Epidermal Cell No

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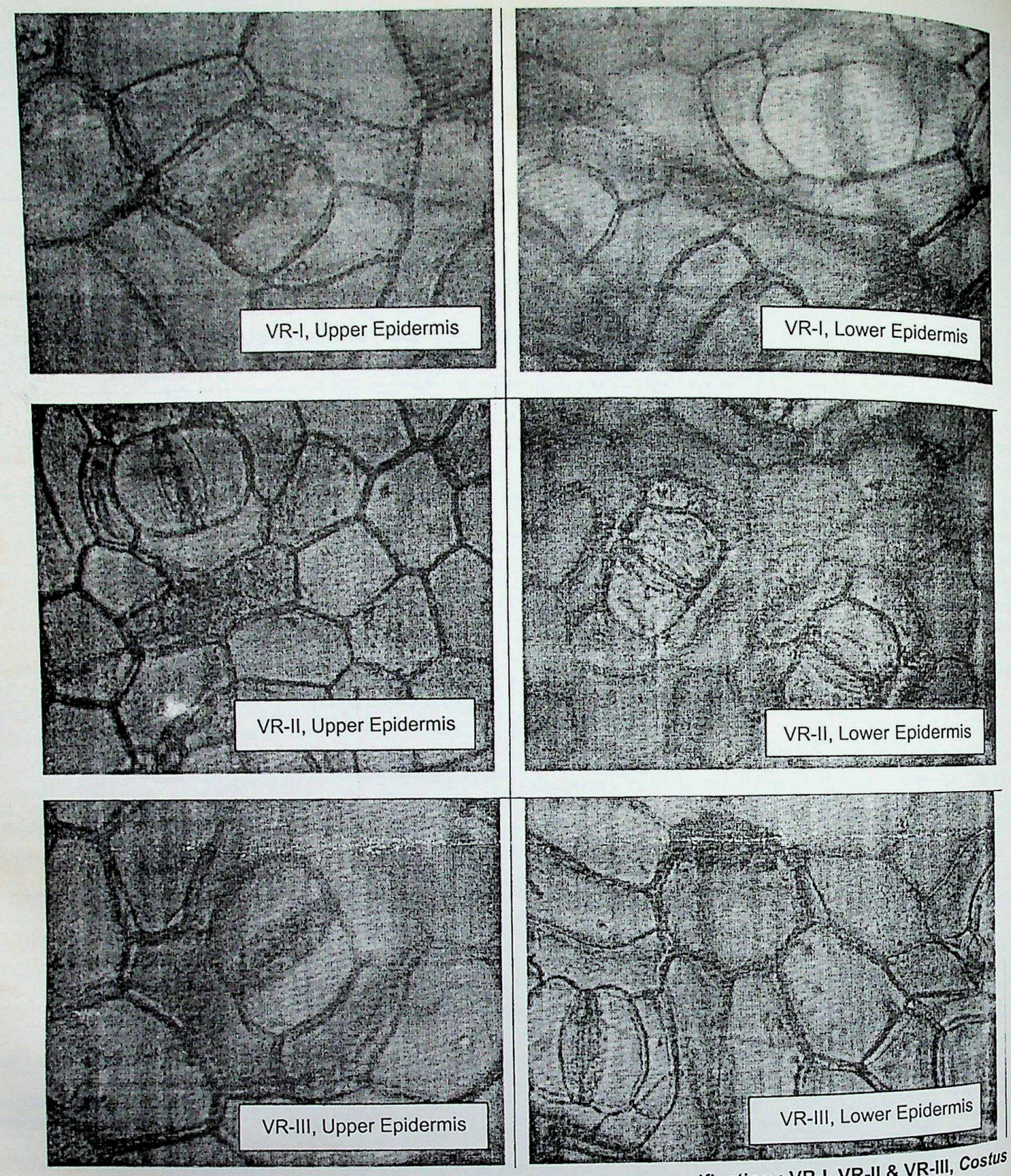

(B) Leaf-epidermal Structures Microphotographs under High Power magnification : VR-I, VR-II & VR-III, *Costus speciosus* (Koenig) Sm.

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Seasonal Effect on the Silkworm Rearing by using Improved Mulberry Varieties

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Studied carried out in different seasons of a year showed non-significant effect to the economic characters of silkworm like single cocoon weight, single shell weight, shell ratio %, leaf cocoon ratio, denier except filament length by using improved mulberry varieties.

INTRODUCTION

Mulberry leaf is the only source of nutrition for the silkworm *Bombyx mori* L., and 70% of the protein content of raw silk is directly derived from the protein of the mulberry leaf. The nutrient contents of mulberry leaves are known to vary according to the season and variety of mulberry (Sarkar *et al.*, 1992; Giridhar.,1996). There is a positive relationship between breeds and variety of mulberry leaves with regard to the expression of most of the cocoon characteristics. The poor nutrient content in improperly matured leaves might have debilitated the silkworm and led to poor cocoon quality (Radha *et al.*, 1988; Susheelamma *et al.*, 1989). Seasonal differences in the performance of different silkworm breeds have been reported (Nataraju *et al.*, 1981).

The importance of quality of leaf on growth, development and health of silkworm has been greatly stressed by Yokayama (1963). Tayade *et al.* (1988) observed that single cocoon weight, shell percentage, yield of cocoons varies when the silkworm larvae were fed on different mulberry varieties.

MATERIAL AND METHODS

In order to evaluate the nutritional status of the leaves of six different experimental varieties (S-13,S-34, S-36,S-1 and V-1) and the control K-2, bioassay analysis through rearing traits were done using the breed (KA x NB4D2) of silkworm *Bombyx mori* L., in different seasons *i.e.*, rainy, winter and summer by adopting Krishnaswamy method (1978b) and collected data of cocoon weight (g), shell weight (g), shell ratio(%) (Harada, 1961), leaf cocoon ratio(:), filament length (m) Mano 1992, denier(d), Nanavathy 1965 and Panes and Sukhatme, (1967) method was adopted for statistical analysis.

RESULTS AND DISCUSSION

The rearing performance of KA x NB4D2 for three different seasons of the year for the economic traits of silkworm, single cocoon weight, shell weight, shell ratio, leaf cocoon ratio, filament length, denier, leaf cocoon ratio are presented in the Table 1 indicated that interaction between the season x variety x hybrid.

Varietal effect on economic characters of cocoons : Results on the effect of five promising improved varieties in comparison with check variety (Kanva-2) on the rearing performance with popular bivoltine hybrid KA x NB4D2 showed considerable variations on varietal effect on economic characters of cocoons. It was observed that silkworm larvae reared on S-13 and V-1 improved varieties recorded significantly higher shell weight, shell ratio, filament length. These results were in confirmation with the findings of Krishnaswamy *et al.* (1970), Verma and Kushawaha (1970), Venugopal Pillai and Jolly (1985), Bari *et al.* (1985), Susheelamma *et al.* (1988, 1989) and Patil *et al.* (2001).

Leaf cocoon ratio in case of V-1,S-1 and S-13 varieties fed to silkworm was less in comparison to Kanva-2 variety (check). This was due to effect of qualitative traits of improved varieties, the similar observations have been made by the Susheelamma *et al.* (1988).

Seasonal effect on different mulberry varieties on economic characters of cocoons : The investigations on the effect of season on different improved mulberry varieties on silkworm breeds KA x NB4D2 revealed that different seasons have significant effect on economic characters such as single cocoon weight, shell weight, shell ratio, leaf cocoon ratio, filament length and denier. Variety x season interaction was highly significant. In respect of different cocoon characters, different varieties of mulberry responded differently in different seasons of the year. Similar observations have been made by Krishnaswamy *et al.* (1970), Bari *et al.* (1985) and Susheelamma *et al.* (1989) where in they reported that interaction between variety and season was highly significant in respect of different cocoon characters and improved mulberry varieties which responded differently in different seasons. The results indicate that S-13 and V-1 improved varieties were superior to other varieties in terms of qualitative assessment.

Effect of different seasons on varieties with different hybrids found was non-significant for all the important economic

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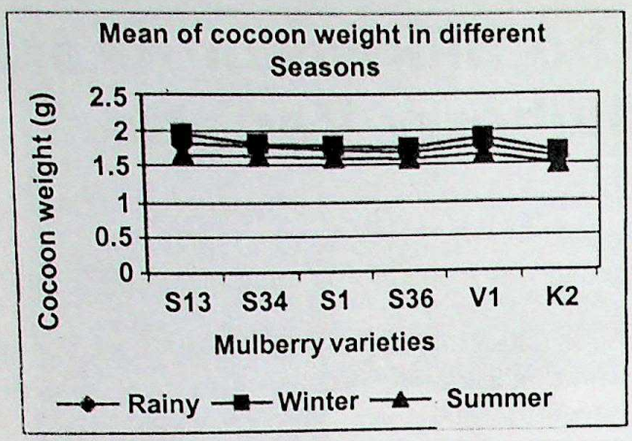

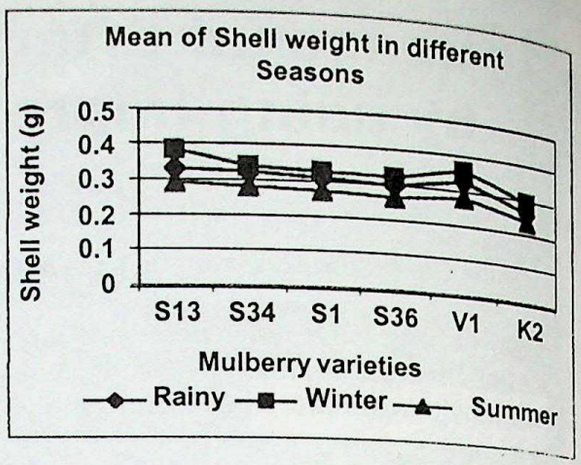

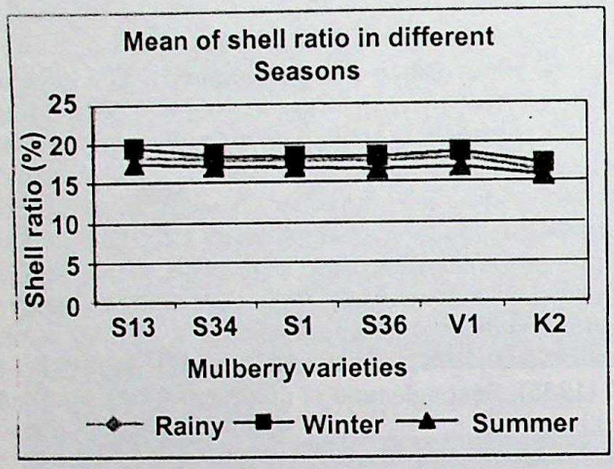

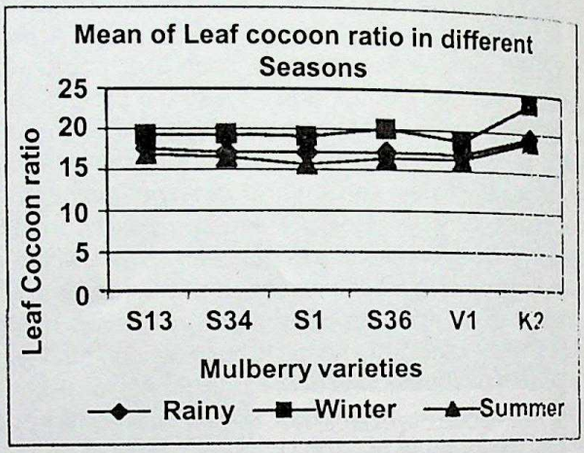

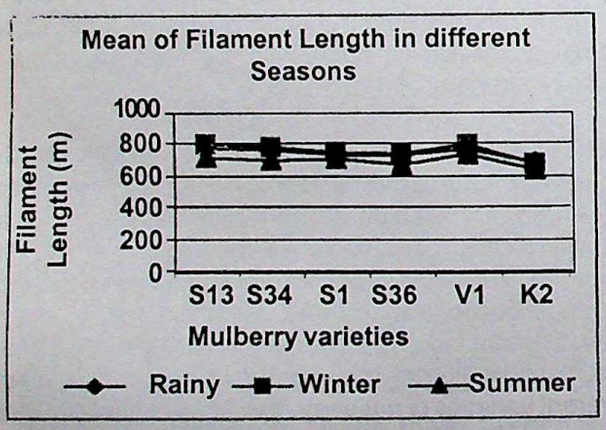

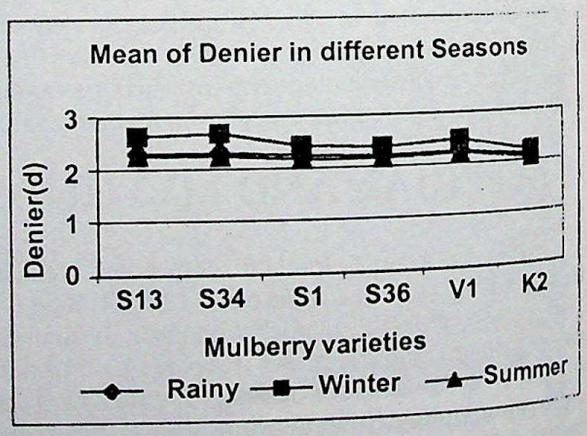

Fig. 1 : Seasonal effect on silkworm rearing with improved mulberry varieties

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Table 1 : Analysis of variance of economic characters of silkworm *Bombyx mori* L for interaction (Season x hybrid x variety).

Source of variations	Degree of freedom	Single cocoon weight	Single shell weight	Shell Ratio %	Leaf cocoon ratio	Filament length	Denier
Season	2	0.467	0.0532	33.986	144.28	44166.04	0.8986
Hybrid	1	0.013	0.0814	225.60	269.64	1779556.00	10.432
S x H	2	0.003	0.0001	0.274	0.605	2879.77	0.049
Varieties	5	0.1522	0.0200	17.565	56.499	62212.61	0.275
S x V	10	0.0177	0.0013	0.232	2.035	1769.45	0.024
H x V	5	0.010	0.0008	1.339	2.411	7014.23	0.007
S x H x V	10	0.006	0.0002	0.155	0.941	1082.02	0.008
Error	108	0.003	0.0001	0.097	0.492	396.55	0.007
CD at 5%							
Season		0.023	0.004	0.126	0.283	8.05	0.034
Race		0.019	0.004	0.102	0.231	6.57	0.0281

characters. This was clearly indicated that season x variety x breeds effect was non-significant and the varietal effect remains same with different hybrids in different seasons of the year.

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Influence of Solar Energy Trap Environment on Seed Germination and Seedling Growth of Brinjal (*Solanum melongena*) and Chillies (*Capsicum annum*)

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The effect of solar energy trap on seed germination, seedling growth, chlorophyll content and mineral status of seedlings have been described, In *Solanum melongena* germination under solar energy trap was increased to 110.00% and that of *Capsicum annum* to 104.87%. The length, fresh weight and dry weight of epicotyls and radicle were also increased in solar energy trap conditions. The epicotyl accumulates 133.34%. 130.76% and radicle 146.15%, 120% more dry weight in brinjal and chilly respectively. The nitrogen, phosphorus and chlorophyll content in the epicotyls and radicle were also influenced.

Keywords : *Capsicum annum, Solanum melongena.* Solar energy trap.

INTRODUCTION

A seed contains miniature plant with suspended growth and low rate of respiration and other activities. It germinates involving four groups of processes *i.e.,* imbibitions, formation of enzyme systems, the commencement of growth of seedlings with the characteristic features associated with the subterranean plant up to the time of emergence from the soil. These processes directly depend upon various environmental factors especially on temperature, humidity and intensity of light. Shukla *et al.,* (1988) reported that the environmental conditions could be controlled in polythene house, which may be favourable for seed germination. Singh *et al.,* (1970) the effect of alkathene covers on germination and seedling growth of Citrus.

The effect of polythene cover conditions on seed germination of different crops has been studied by Hopen and Ries (1962), Wells and Brentloy (1985), Behera *et al.,* (1990), Puspangadan *et al.,* (1984), Varga (1984), Matsubara and Masuda (1984), Somos (1984) and Sirohi (1994). In the paper, the effect of solar energy trap on seed germination. seedling growth, chlorophyll content and mineral status of seedlings have been described and analysed.

MATERIAL AND METHODS

Morphologically similar seeds of *Solanum melongena* and *Capsicum annum* were selected sterilized, with 0.1 per cent $HgCl_2$, rinsed thoroughly with distilled water and soaked in water for 12 hours. After soaking 50 seeds of each lot were transferred to wet filter papers in petri plates for germination in solar energy trap and in open field conditions. The radicle emergence (2 mm) was taken as criterion for germination. For seedling growth measurement, seedlings were allowed to grow in both the conditions, *i.e.,* open field and solar energy trap. Eight-day old seedlings were dissected into epicotyl and radicle for growth measurement studies. Chlorophyll pigments in leaves were measured spectrophotometrically (Koski and Smith, 1948). Total Nitrogen was determined following the method given by Piper (1942) and total phosphorus by the method of Allen (1940).

RESULTS AND DISCUSSION

The data presented revealed that the rate of germination and seeding growth was faster in solar energy trap conditions as compared to the normal environment conditions.

In *Solanum melongena* germination in solar energy trap was increased to 110.00 per cent of the control. The length, fresh weight and dry weight of epicotyl were increased to 112.18, 126.97 and 133.34 per cent respectively. The length of the radicle, fresh weight and dry weight were also increased to 109.06 107.79 and 146.15 per cent of the control respectively. Nitrogen and phosphorus contents were changed to 99.81 and 103.11 per cent in epicotyl and 93.01 and 98.89 per cent in radicle under solar energy trap environment over normal. Chlorophyll 'a' increased to 101.25 per cent while chlorophyll 'b' and protochorophyll increased to 105.55 and 82.00 per cent respectively.

In *Capsicum annum* increased to 104.87 per cent in solar energy trap conditions, The length, fresh weight and dry weights of epicotyl were increased 114.06, 120.54 and 130.76

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Table 1 : Influence of solar energy trap environment on seed germination and seedling growth of *Solanum melongena*.

Parameters	Control Seedlings	Seedlings raised in the Solar Energy Trap Environment	Variations over Control (%)
Germination (%)	80.00	88.00	110.00
Epicotyl			
Length (cm)	2.71± 0.29	3.04 ± 2.89	112.18
Fresh Wt. (mg)	89.00 ± 3.74	113.00 ± 2.89	126.97
Dry Wt. (mg)	9.00 ± 1.05	12.00 ± 2.55	133.34
Redicle			
Length (cm)	3.42 ± 0.38	3.73 ± 0.49	109.06
Fresh Wt. (mg)	77.00 ± 3.42	83.00 ± 3.15	107.79
Dry Wt. (mg)	6.50 ± 0.70	9.50 ± 0.81	146.15

± - standard deviation.

Table 2 : Influence of solar energy trap environment on seed germination and seedling growth of *Capsicum annum*.

Parameters	Control Seedlings	Seedlings raised in the Solar Energy Trap Environment	Variations over Control (%)
Germination (%)	82.00	86.00	104.87
Epicotyl			
Length (cm)	3.20 ± 0.28	3.65 ± 0.48	114.06
Fresh Wt. (mg)	112.00 ± 2.17	135.00 ± 2.01	120.54
Dry Wt. (mg)	13.00 ± 1.10	17.00 ± 2.15	130.76
Redicle			
Length (cm)	4.08 ± 0.30	4.24 ± 0.60	103.92
Fresh Wt. (mg)	101.00 ± 2.75	112.00 ± 3.12	110.89
Dry Wt. (mg)	9.00 ± 0.90	10.80 ± 0.81	120.00

± - standard deviation.

Table 3 : Influence of solar energy trap environment on physiological and biochemical aspects of *Solanum melongena* seedlings.

Parameters	Control Seedlings	Seedlings raised in the Solar Energy Trap Environment	Variations over Control (%)
Biochemical Aspects:			
Total Nitrogen (mg gdw^{-1})			
Epicotyl	52.10	52.99	99.81
Radicle	38.60	35.90	93.01
Total Phosphorus (mg gdw^{-1})			
Epicotyl	6.10	6.29	103.11
Radicle	8.10	8.01	98.89
Physiological aspects:			
Chlorophyll (ng gdw^{-1})			
Chl. 'a'	0.95	0.96	101.25
Chl. 'b'	0.36	0.38	105.55
Protochlorophyll	0.17	0.14	82.00

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Table 4 : Influence of solar energy trap environment on physiological and biochemical aspects of *Capsicum annum* seedlings.

Parameters	Control Seedlings	Seedlings raised in the Solar Energy Trap Environment	Variations over Control (%)
Biochemical Aspects:			
Total Nitrogen (mg gdw^{-1})			
Epicotyl	50.50	56.10	111.09
Radicle	45.10	48.20	106.87
Total Phosphorus (mg gdw^{-1})			
Epicotyl	6.00	6.90	113.11
Radicle	6.20	6.10	98.38
Physiological aspects:			
Chlorophyll (ng gdw^{-1})			
Chl. 'a'	0.90	0.92	102.22
Chl. 'b'	0.29	0.25	86.21
Protochlorophyll	0.13	0.15	115.38

per cent, respectively. Similarly the radicle length, fresh weight and dry weight increased to 103.92, 110.89 and 120.00 per cent respectively in solar energy trap conditions. Nitrogen and phosphorus contents also increased to 111.09 and 113.11 per cent in epicotyl, 106.87 and 98.38 per cent in radicle under solar energy trap conditions. These conditions also improved the chlorophyll content of the leaves in seedling stage. Chlorophyll 'a', chlorophyll 'b' and protochlorophyll contents increased to 102.22, 86.21 and 115.38 per cent of the control respectively.

Better germination and growth of seedlings in the solar energy traps are mainly due to the cumulative effect of the environmental factors, like temperature, humidity and light intensity (Sirohi, 1994). The increase in the seedling length may be due to a decrease in light intensity. The increase in the production of dry matter reveals increased photosynthetic efficiency inside the solar energy trap, which is apparent from the increasing chlorophyll pigments. Decreasing nitrogen and phosphorus contents suggest that the mineral absorption is lower at the seedling stage.

Similar results have been obtained by Singh *et al.* (1970) in Citrus, Naudet (1986) in beech, Behera *et al.*, (1990) in paddy, vegetables and flowers, Puspangadan *et al.*, (1984) in *Ocimum gratissimum* cv. *Clocimum*, Shinde *et al.*, (2005) in carnation, Varga (1984) in paprika, Kaminksi (1982) in *Abies grandis*, Somos (1984) in the seedlings of various vegetables, Kumar and Goyal (1995) in legumes and Sirohi (1994) in cucurbits.

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Studies on the Integrated Management of Pest and Diseases in Mulberry (*Morus alba* L.) in Black Cotton Soils with Organic Products and Botanical Extracts

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Field experiments were conducted in a Randomized Block Design (RBD) at the mulberry farm of Research Extension Centre (REC), Vikarabad (A.P) during the year 2006-08 to assess the effect of certain organic formulations and botanical extracts in controlling the pest and diseases of foliage and root diseases of V-1 mulberry (*Morus alba* L.) variety in three seasons, *viz.*, summer, rainy and winter and available soil nutrients and micro-flora. The results of the farm trial revealed that among the nine treatments T_6 has recorded significanlty higher reduction of percentage of disease intensity (PDI) in foliar pest and diseases as well as root diseases as compared to other treatments, but it did not differ with T_7. The soil physical (pH, EC and OC), chemical (N,P,K,Ca, Mg, Mn, S, Cu, Fe and Zn) nutrients and microbial populations (bacteria, fungus, actinomyceties and *Azotobacter*) at leaf harvest (65-70 DAP) also improved significantly with T_6 and T_7 as compared to their initial status of other treatments but did not differ each other.

Keywords : Mulberry, Pests and Diseases, Organics, Botanical extracts, Soil nutrients, Black Cotton Soil.

INTRODUCTION

Mulberry (*Morus alba* L.) is a fast growing perennial tree and it is cultivated as bush, middle bush and tree for its valued leaf for silkworm (*Bombyx mori* L) rearing. It is affected by several pathogenic diseases caused by fungi, bacteria, mycoplasma and virus (Sengupta *et al.*, 1990). Foliar diseases *viz.*, powdery mildew (*Phyllactinia corylea*), leaf spot (*Cercospora moricola*), leaf rust (*Cerotelium fici*) and leaf blight (*Pseudomonas mori*); foliar pests *viz.*, tukra (*Maconellicoccus hirsutus*), Jassid (*Empoasca flavescens*), thrips (*Pseudodendrothrips mori*), leaf roller (*Diaphania pulverulentalis*), bihar hairy caterpillar (*Spilosoma obliqua*) and root diseases, *viz.*, root-knot (*Meloidogyne incognita*) and root-rot (*Fusarium solani* & *Fusarium oxysporum*) are the important mulberry diseases in Southern region of India (Tomy Philip *et at.*, 1997). This pest and diseases cause 10 - 30% loss in leaf yield in terms of destruction of leaf lamina, immature leaf fall and depletion in nutritive value (Sengupta *et al.*, 1990). Indiscriminate use of chemical fertilizers insecticides in mulberry cultivation resulted in pest resistance, resurgence, secondary pest out break and reduction in biodiversity of natural enemies besides environmental pollution (Van Lenteren and Woets, 1988). In view of recent tirade against the use of synthetic fungicides and pesticides efforts are underway to search economically viable, non-toxic biodegradable phyto-extracts and animal by-products to utilize for the control of mulberry pest and diseases and enhancement of soil nutrition status. Hence, the present study was undertaken to study the integrated impact of organic products and botanical extracts done with cow urine, on controlling the pest and diseases of foliage and root diseases of mulberry done in three seasons *viz.*, summer, rainy and winter and available nutrient and microbial population status at initial and at leaf harvest of crop in black cotton soil.

MATERIAL AND METHODS

The farm was with black cotton soil. The initial physical (pH, EC and OC), chemical (N,P,K,Ca, Mg, Mn, S, Cu, Fe and Zn), and microbial (bacteria, soil fungus, actinomyceties and *Azotobacter*) status of experimental soil is being presented in Tables-5 and 6. The field was prepared well and at the last ploughing farm yard manure (FYM) was applied (20 t ha^{-1}) and incorporated. The experiment was conducted in randomized block design (RBD) with three replications in V-1 mulberry plantation raised in 90cm x 90cm spacing. The treatments in experimental site were *viz.*, T_1- Control (100 % NPK recommended dose @ 350:140:140Kg NPK ha^{-1}, T_2-75% RDF (265:105:105: Kg NPK ha^{-1}) + GM (Green manure,

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Daincha) + CFS (Chemical foliar spray), T_3-50% RDF (175:70:70 Kg NPK ha^{-1}) + GM (Green manure, Daincha) + CFS (Chemical foliar spray), T_4- 25% RDF (88:35:35 Kg NPK ha^{-1}) + GM (Green manure, Daincha) + CFS (Chemical foliar spray), T_5- 0% RDF + GM (Green manure, Daincha) + CFS (Chemical foliar spray), T_6-75% (RDF(265:105:105 Kg NPK ha^{-1}) + JVT (Jeevamrutha) + GM (Green manure, Daincha) + BFS (3% Botanical extracts foliar spray and soil drench), T_7-50% RDF (175:70:70 Kg NPK ha^{-1}) + GM (Green manure, Daincha) + JVT (Jeevamrutha) + BFS (3% Botanical extracts foliar spray and soil drench), T_8-25% RDF (88:35:35Kg NPK ha^{-1}) + GM (Green manure, Daincha) + JVT (Jeevamrutha) + BFS (3% Botanical extracts foliar spray and soil drench), T_9-0% RDF + GM (Green manure, Daincha) + BFS (3% Botanical extracts foliar spary and soil drench). Irrigation was provided through drip system to all the treatments, except in rainy days.

Preparation of Plant Extracts : Phyto-extracts of twenty plant materials (Table-1) was prepared by homogenation of 800g of clean and washed plant materials (leaf/seeds/bulbs/rhizome) @ 40g each in electrical blender using fresh cow urine @ 2ml g^{-1}. The extract was filtered through two layers of moistened muslin cloth. The plant extracts so prepare were centrifuged at 3000 rpm for 15 minutes. The final volume was adjusted to 1000ml fresh cow urine and maintained as stock extract. The stock extract was further diluted to desired concentrations with fresh water before use.

Preparation of Jeevamrutha: 20 liters of cow's urine was collected in to 200lt. capacity plastic drum and added 20 Kg of fresh cow dung, 2 Kg of legume seeds flour, 2 Kg of jaggery and handful virgin soil (100 g) of boundary bund of the test plot and made up to 200 lt. with freshwater and capped air tight and allowed the mixture for 5-6 days to decomposition. Thrice in a day the mixture was stirred well clock wise direction for 20 minutes. To obtain best results it should be utilized with in fortnight after preparation of this biological mixture.

Methodology adopted: Each experimental plot had 27 sub-plots and each sub-plot was with 56 plants. Fifteen days after pruning of sub-plots, Daincha, *Seshania aculeata* (N-2.5%, P-0.40% and K-1.29%, S-0.57% and Zn-29 ppm) @ 4t per acre was soil mulched (*ex-situ*) in 90cm x 90cm spacing followed by flood irrigation through bore well and then for entire crop period drip irrigation was provided. Jeevamrutha was applied to plots through fertigation tank of drip irrigation system five days after mulching of green manure, as first dose, and second dose was given fifteen days after first dose. 3% botanical extracts (3 ml/ l.of fresh water) was sprayed on mulberry foliage followed by soil drenching 20 days after pruning, as first spray/ drench and after ten days interval of first spray second spray/ drench was given for treatment plots. Chemical foliar sprays for control of pest and diseases and basal doses of chemicals for control of root diseases were used as recommended by Tomy Philip *et al.*, 1994.

Foliar diseases incidence (DI) was calculated randomly in each treatment plot. The infected leaves were then categorized in to 5 grades as follows. Grade-1: no incidence; grade-2: up to 5% leaf area infected; grade-3: 5 – 20% leaf area infected; grade-4: 20 - 50% leaf area infected and grade-5: above 50% leaf area infected. The per cent disease index (PDI) was calculated based on the following formula (McKinney, 1923).

$$PDI = \frac{\text{Sum of category values}}{\text{Total number of leaves observed x maximum grading (5)}}$$

The incidences of foliar pests and root diseases were calculated by using the following formula.

$$\text{Pests \& Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

The available soil status of pH, EC, OC, N, P, K, Ca, Mg, S, Mn, Cu, Fe and Zn at a depth of two feet at leaf harvest (65-70 DAT) stage was recorded by following standard method of soil analysis (Jackson, 1973; Perur *et al.*, 1973). The number of microflora and fauna was also calculated (Cappuccino and Sherman, 1983). The data were statistically analyzed for ANOVA (Snedecor and Cochran, 1967) (Table - 2 to 6).

RESULTS

The perusal of results (Tables 2 - 6) revealed the incidence of all foliar pest and diseases as well as root diseases were significantly reduced due to basal (soil) and foliar application of organics along with the various doses of chemical fertilizers. The best result, however, was recorded with the treatment T_6 followed by the treatment T_7 and other treatments. The difference in the incidence of pests and diseases of foliage and root diseases between T_6 and T_7 treatments was insignificant. The other treatment plots, which graded levels of chemical fertilizers along with the organics and chemical foliar spray (CFS) recorded significantly lesser incidence of pest and diseases of foliage and root diseases when compared to the control plot, which received 100% chemical fertilizers only. Similarly, the soil status of physical properties, chemical nutrients and micro-flora has significanlty enhanced due to the treatment T_6 as compared to other treatments, but has differed insignificantly with T_7.

DISCUSSION

The maximum reduction in the occurrence of pest and diseases of foliage and root diseases of mulberry, as well as increase of soil physical, chemical and biological status were recorded with the treatment combinations made with Jeevamrutha (basal application), green manure (soil mulch) and botanical extracts (foliar spray and soil drench) (Table-2-6).

Effect of integrated organics on incidence of foliar diseases : The basal application of FYM green manure and Jeevamrutha together with chemical fertilizers enhanced soil physical condition, nutrients availability in soil media and their higher uptake in plants might be the reason for triggering of

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the physiological activity to resist to pathogens in soil and foliage of plants. The botanical extracts done with cow's urine used as foliar spray on mulberry leaves and soil drench significantly reduced the foliar diseases might be due to its anti-fungal properties. The fungi-toxicity of extracts of tropical plants observed in this study is in consonance with the earlier investigations. Balasubramaniam *et al.*, (1996) reported that the paddy soaked in diluted cow's urine before sowing has considerably reduced the incidence of leaf spot and rice blast diseases. Sabillion and Bustamanta (1995) reported that amongst all available botanicals, neem based products were found effective against both diseases and pests. Sarvamangala *et al.*, (1993) reported the inhibition of the leaf extracts of neem and parthenium on the germination of *Peridiospora mori* (mulberry leaf rust) and *Cercospora moricola* (mulberry leaf spot). Biswas *et al.*, (1995) found that *A. indica* and *Adhatoda zeylanica* were effective in reducing powdery mildew disease in mulberry. There are three possible modes of action of plant extracts in controlling the diseases *viz.,* 1). Anti-pathogenic action of phytochemicals, 2). Growth promotion of beneficial microorganisms (antagonistic to pathogents) on the host plant (elicitor action).

Effect of integrated organics on incidence of foliar pests : The maximum reduction in occurrence of insect-pests species might be due to the integrated defensive mechanism of phenol contents in organic manures, alkaloid contents in plant extracts and anti-feedant and repellant nature of cow urine used in this study. Effect of cow's urine alone and together with plant extracts was reported by few workers. The incidence of mustard aphid was managed by adopting 3 or 4 foliar sprays of neem kernel extract in cow urine (3%) either alone or in combination with reduced dose of Dimethoate *i.e.,* 0.03% (Gupta, 2005). Cow's urine repelled whiteflies, *Bemissia tabacci*, in cotton crop (Hecht and Badiane, 2003). Tesfaye and Gautam (2003) reported that fermented cow urine (1:6) was effective in reducing the bark aphid, *D. noxia* and Welo bush cricket, *D. brevipennis*, and it was at par with treatment with other natural extracts, *viz.*, tobacco (*Nicotiana tobacum*), garlic (*Allium cepa*), neem (*Azadirachta indica*) and chilli *(Capsicum annuum)*. Mortality of the banana fly, *D. melanogaster* treated with 3 days fermented cow urine (1:6) resulted in 79.6% mortality as against 2.8% in control and was statistically at par with garlic and 3 times more effective than 5% NSKE (Tesfaye and Gautam, 2003). Parkman and Peinkowski (1990) reported that soil drenches of 1.0 and 2.0 ppm azadiractin applied to infested Chrysanthemum (*Odendranthema morifolium*) significantly reduced female fecundity and longevity of males was reduced by 2.0 ppm drench treatment. Primary effects include the process of chemo-respiration by the organism *e.g.*, sensory organs on mouth parts, which stimulate the organism to begin feeding, where as secondary processes are effects such as gut mortality disorders due to topical application only (Ascher, 1993).

Effect of organics against incidence of root diseases: The incidence of root-rot and root-knot diseases was significantly reduced with basal organics and foliar spray and soil drench with botanical extracts made with cow urine. This might be due to anti-fungitoxic and nematicidal (alkaloids) properties of the soil amendments and botanical extracts. Further, Jeevamrutha could have inhibited the proliferation of soil pathogens and improved the soil health with supplementation of required nutrients, growth promoting substances and beneficial microbes as observed in this study (Table-5 and 6). Added to that the botanical extracts sprayed on the mulberry plants and soil drench might be absorbed by the leaves and transported to the root system, which resulted is suppression of the root pathogens. Luo and Zhaoxuan (1989) have observed the suppression of root-rot disease, *Fusarium* spp. and growth of mulberry plantation by using organic manures and botanical foliar sprays. Alam (1993) reported the significant reduction in the number of knots, egg masses and nematode population due to application of neem and pongamia kernel extracts, which produce strong volatile/alkaloid compounds such as nimbidin, theonemone and phenols. Luo and Zhaoxuan (1989) have observed the suppression of root-rot disease, *Fusarium* spp. and growth of mulberry plantation by using organic manures and botanical sprays. Inhibition of radial mycelial growth of *F. solani* and *F. oxysporium* by the leaf extracts of neem (*A. indica*) and Karanj (*Pongamia glabra*) was observed through *in-vitro* study by Tomy Philip *et al.* (1997). The soil drenching with leaf extracts of piper against *P. monospermum, E. mirotheca* and *P. aphanidermatum* (Narayana Bhat *et al.*, 1990) was effective. Kovacs (1964) reported that *Fusarium lini* infection of flax seeds and *R. solani* infection of lettuce seeds were inhibited by applying onion extracts to infested soil. Thus, the anti-fungal and anti-nematicidal properties of plant extracts done with cow's urine, used as soil drench and Jeevamurtha, used as soil application might have inhibited the respiration metabolism through the presence of respiration inhibitor, carboxine, which interfered with the synthesis of protein DNA and RNA in rapidly metabolizing the cell of the organism (Natarajan and Lalithakumari, 1987).

Physico-chemical and micro-flora status in soil

Physico-chemicals : The pH, EC, and OC values recorded in the soil amended with organics, at leaf harvest time showed significant improvement over the initial status of soil. The reduction of pH was much pronounced over the initial values, might be attributed to the formation of organic acids during decompostion of organic matter. There observations are in line with those of Sushma *et al.* (2007). Like, pH the increase in soil EC and OC contents at leaf harvest might be due to continuous *ex-situ* of *S. aculeata* and addition of decomposed FYM and Jeevamurtha. These results are corroborated with the findings of Sushma *et al.*, (2007).

Organic manures, green manure and inorganic fertilizers might have been solubilized in soil due to acidifying action of different micro-organisms during decomposition and made available of more NPK. The green manure undergoes slow decomposition and the mineral mobilisation may help in the release of 'N' to meet the requirement of plants at crucial stage (Meelu *et al.*, 1986). Increase in available 'P' might be due to organic matter added, that would be attributed to enhance the activity of phosphorous solubilizing micro-organisms with organic matter (Dhoke *et al.*, 2007. The treatment cobination with *ex-situ* of *S. aculeata* registered

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higher content of 'K' availability could be due to higher nutrient content in it and easy decompostion of mineral constituents and their effect in dislodging the exchangeable K into solution (Meelu *et al.*, 1986). Available magnesium (Mg) and manganese (Mn) contents of the soil were significantly high in the plots treated with organic formulations might be due to the addition of green manure through mineralization and release of native Mg and Mn (Duraiswamy and Mani, 2002). Available sulphur (S) content was significantly higher in the soil media could be due to addition of organic manures with green manure, *S. aculeata*, continuously, enhanced the 'S' availability in the soil (Srikanth *et al.*, 2000) owing to its' 'S' content. The highest iron (Fe) Copper (Cu) contents were found in the organically treated soils. The release of Fe, Cu and Zn to available pool in soil could be due to the action of organic acids produced from organic manures, as this treatments contain considerable amount of Fe, Cu and Zn. Similar observations were reported by Sushma *et al.* (2002).

Micro-flora: The micro-flora status of soil (Table-6) at leaf harvest significantly increased as compared to initial level, could be due to *ex-situ* decomposition of green manure together with the added organic manures and graded levels of chemical fertilizers. The results are in the line with the findings of Amanullah (1997) in Cassava, which revealed that application of organic manures and *ex-situ* composted green manure increased the friability, aggregation and level of humus in soil, thereby enhancing the microbial activity and synthesis of phytohormones necessary for the plant development and resistance to diseases. Nieto and Frankenberger (1989) also reported increased cytokinins in the rhizosphere due to organic manure amendments, which in turn increased rhizosphere micor-flora.

CONCLUSION

The study demonstrated the effectiveness of organic formulations (FYM, Jeevamurtha, BFS (Botanical extracts, as foliar and soil drench) and *in-situ* composted green manure, (*S. aculeate*) in controlling the pests and diseases of mulberry and enhancing the physical, chemical and biological status of soil media consistently all along the crop period. Hence, it signifies that this approach will be very much useful for integrated pest (IPM), diseases (IDM) and nutrient management (INM) in mulbery cultivation.

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Table 1 : List of Medicinal Plants used for the control of pest and diseases of Foliage and root diseases in mulberry.

Sl. No.	Botanical Name	Family	Part used
1.	*Adhatoda zylanica* Ness	Acanthaceae	Leaf
2.	*Allium sativum* Linn.	Liliaceae	Bulb
3.	*Aloe vera* L.	Liliaceae	Leaf
4.	*Annona squamosa*	Annonaceae	Leaf
5.	*Azadirachta indica* L.	Meliaceae	Leaf
6.	*Calotropis gigantea*	Asclepiadaceae	Leaf
7.	*Cassia siamia*	Caesal piniaceae	Leaf
8.	*Croton roxburghii* Black	Euphorbiaceae	Leaf
9.	*Cucumis sativum* Linn.	Cucurbitacease	Rhizome
10.	*Curcuma domestica*	Zingiberaceae	Rhizome
11.	*Datura metel* Linn.	Solanaceae	Leaf
12.	*Eucalyptus globules* Labill	Myrtaceae	Leaf
13.	*Euphorbia hirta* Linn.	Euphorbiaceae	Leaf
14.	*Jotropha curcua* Linn.	Euphorbiaceae	Leaf
15.	*Melia azedarach* Linn.	Meliaceae	Leaf
16.	*Ocimum sanctum* Linn.	Lamiaceae	Leaf
17.	*Prosopis cineraria* Linn.	Mimosaceae	Leaf
18.	*Vitex negudo* Linn.	Verbenaceae	Leaf
19.	*Zingiber oxyphyllium* Rose	Zingiberaceae	Rhizome
20.	*Ziziphus jujube* Mill	Phamnaceae	Leaf

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Table 2 : Effect of organics on incidence of foliar diseases of mulberry at farm level (Average of 10 crops).

Treatment	Leaf Spot			Leaf Rust			Leaf Blight			Powdery mildew		
	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter
T-1	10.65	19.85	3.50	1.00	2.50	8.50	22.00	10.50	1.50	0.0	2.50	23.50
T-2	7.99	4.65	2.28	0.90	0.35	3.50	6.75	4.60	1.00	0.0	0.35	4.29
T-3	7.95	4.95	1.45	0.28	0.30	4.50	5.45	4.75	1.00	0.0	0.35	4.30
T-4	6.96	4.96	1.45	0.28	0.30	4.02	6.45	3.25	1.05	0.0	0.30	4.32
T-5	7.00	4.25	1.30	0.15	0.32	3.68	6.85	3.15	1.00	0.0	0.30	3.32
T-6	0.25	0.05	0.05	0.00	0.02	0.02	0.99	1.00	0.00	0.0	0.00	1.09
T-7	0.25	0.08	0.08	0.00	0.02	0.10	1.02	1.10	0.00	0.0	0.00	1.09
T-8	0.29	1.00	0.09	0.30	0.32	0.10	1.20	1.10	0.00	0.0	0.02	1.00
T-9	2.90	2.00	2.00	0.40	0.33	0.20	1.09	1.20	1.00	0.0	0.04	1.90
Mean	**4.92**	**4.64**	**1.36**	**0.37**	**0.50**	**2.74**	**5.76**	**3.41**	**0.73**	**0.0**	**0.43**	**4.98**
CD at 5%	**2.50**	**3.50**	**1.50**	**0.25**	**0.30**	**2.60**	**3.50**	**3.0**	**1.25**	**0.0**	**1.28**	**5.20**

Table 3 : Effect of organics on incidence of insect-pests of mulberry at farm level (Average of 10 crops).

Treatment	Tukra			Thrips			Jassids			Leaf Roller			Bihar hairy caterpillar		
	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter
T-1	20.80	15.20	4.50	45.50	10.50	2.50	20.30	8.50	2.00	0.0	35.50	10.0	10.50	3.50	2.50
T-2	6.48	3.95	3.19	3.45	3.25	1.22	3.51	2.26	0.70	0.0	5.52	3.22	3.66	1.66	0.44
T-3	5.45	3.94	2.19	3.46	3.20	0.24	3.48	2.26	0.71	0.0	6.50	3.21	4.62	1.65	0.94
T-4	5.48	3.95	2.19	4.45	3.20	0.20	3.49	2.26	0.71	0.0	5.50	3.21	3.06	1.66	0.95
T-5	5.45	3.94	2.10	4.79	4.25	0.30	3.50	2.25	0.70	0.0	4.52	3.20	3.06	1.65	0.99
T-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00	0.20	0.50	0.90
T-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.00	0.20	0.60	0.90
T-8	0.50	0.05	0.20	0.00	0.00	0.00	0.00	0.20	0.70	0.0	0.00	0.00	0.30	0.65	0.95
T-9	1.99	2.00	1.50	0.90	0.00	0.00	0.50	0.25	0.90	0.0	1.20	0.00	0.99	0.90	0.95
Mean	**5.13**	**3.67**	**1.76**	**6.95**	**2.71**	**0.50**	**3.86**	**1.99**	**0.71**	**0.0**	**6.53**	**2.54**	**2.95**	**1.42**	**1.06**
CD at 5%	**2.56**	**1.95**	**1.85**	**3.65**	**2.75**	**1.55**	**2.60**	**3.50**	**2.60**	**--**	**3.65**	**2.50**	**2.50**	**1.75**	**1.25**

Table 4 : Effect of organics on incidence of Root diseases of mulberry at farm level (Average of 10 crops).

Treatment	Root-rot			Root-knot		
	Summer	Rainy	Winter	Summer	Rainy	Winter
T-1	25.80	10.50	1.50	2.50	10.50	3.00
T-2	10.12	4.90	0.52	0.90	3.60	1.38
T-3	10.12	4.91	0.51	0.90	3.50	1.36
T-4	12.11	4.00	0.50	0.90	4.00	1.35
T-5	11.15	4.22	0.60	0.90	3.08	1.45
T-6	2.00	1.00	0.05	0.49	1.20	0.35
T-7	2.10	1.00	0.05	0.50	1.20	0.36
T-8	2.12	1.10	0.05	0.50	1.50	0.38
T-9	3.00	2.00	0.90	0.50	3.00	0.90
Mean	**8.72**	**3.74**	**0.52**	**0.90**	**3.51**	**1.17**
CD at 5%	**5.85**	**3.85**	**1.26**	**1.75**	**2.85**	**1.85**

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Table 5 : Effect of organics on nutrient availability at farm level at leaf harvest (Average of 10 crops).

Treatment	Ending												
	pH	EC	OC	N	P	K	Ca	Mg	S	Fe	Zn	Cu	Mn
		dSm^{-1}	$g\ Kg^{-1}$	$Kg\ ha^{-1}$			Cmol (P) Kg^{-1}			mg Kg^{-1}			
T-1	8.14	0.55	5.49	229	89.0	278	13.2	18.5	40.0	3.90	0.70	0.30	3.80
T-2	8.09	0.54	5.55	250	70.1	280	13.2	19.5	41.5	3.94	0.71	0.31	3.85
T-3	8.01	0.47	6.10	266	71.5	281	13.3	20.0	45.65	3.95	0.71	1.31	3.95
T-4	8.00	0.48	6.40	265	72.2	282	13.2	20.0	49.6	3.95	0.72	1.32	3.95
T-5	8.10	0.54	6.50	275	72.5	300	13.5	20.6	56.2	4.00	0.74	1.30	4.16
T-6	7.00	0.53	6.65	275	85.3	370	14.9	22.9	59.6	4.45	0.78	1.45	4.60
T-7	7.00	0.54	7.25	300	84.5	370	14.9	25.6	59.9	4.24	0.78	1.45	4.59
T-8	7.10	0.53	7.20	295	81.8	369	14.7	24.2	58.8	4.24	0.75	1.43	4.52
T-9	7.50	0.53	6.85	275	75.0	290	13.2	20.0	49.6	3.15	0.65	1.30	4.00
Mean	**7.66**	**0.58**	**6.44**	**270**	**77.99**	**313**	**13.79**	**21.26**	**51.15**	**3.98**	**0.73**	**1.13**	**4.16**
CD at 5%	**NS**	**NS**	**NS**	**28.5**	**2.88**	**10.2**	**1.50**	**0.95**	**9.25**	**NS**	**NS**	**NS**	**NS**
Initial	**8.25**	**0.58**	**5.90**	**208**	**38**	**275**	**2.70**	**21.05**	**37.0**	**3.70**	**0.65**	**1.20**	**3.71**

Table 6 : Effect of organics on microbial population at farm level at leaf hearvet (Average of 10 crops).

Treatment	Bacteria	Fungus	Actinomycetes	*Azotobacter*
	C fu g^{-1} soil x 10^6			
T-1	60.35	30.25	5.50	2.25
T-2	65.50	33.50	7.69	2.19
T-3	65.00	32.00	7.62	2.20
T-4	65.00	32.01	7.61	2.20
T-5	65.80	32.00	7.65	2.20
T-6	69.50	38.00	10.63	3.95
T-7	69.50	36.50	9.75	3.85
T-8	68.50	32.86	9.65	3.80
T-9	67.50	30.50	7.25	2.75
Mean	**66.29**	**33.07**	**8.15**	**2.82**
CD at 5%	**2.85**	**3.00**	**2.65**	**1.85**
Initial	**40.95**	**21.25**	**4.25**	**1.55**

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Morphology and Yield Attributing Features of Brinjal under Solar Energy Trap

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Solar energy trap has played role on morphological improvement of brinjal by providing microclimate that favors growth and productivity of the plant. Growth pattern studies of Brinjal showed that stem length, fresh weight and dry weight of stem, leaf number, flower and fruit number as well as their fresh and dry matter was increased under solar energy trap environment. The flowering was precocious by 4 days and crop matured 10 days earlier under solar energy trap environment as compared to the crop grown under normal environmental conditions.

Keywords : Brinjal, Solar Energy trap, Fresh weight, Dry weight.

INTRODUCTION

Solanum melongena, popularly known as brinjal or eggplant is an economically important fruit vegetable, which has an important role in the human dietary strategy by providing vitamins and minerals. It is a summer crop so for crop improvement and availability of the produce through out the year, during off-season, especially under the mountainous topography and climate conditions use of solar energy trap was made. There has been growing emphasis on growth and productivity of the crop. Total sum of factors (humidity, slightly low light intensity and high temperature) under solar energy trap environment proved favorable for the production of dry matter content in tomato plant (Konyaeva and Korzinnikova, 1983b). However specific attempt has not been made so far to focus on the effect of solar energy trap on root, stem leaves, flowers and fruits in terms of their length, fresh weight and dry weight of brinjal, hence the influence of solar energy trap technology was evaluated for improving the morphology and yield of eggplant.

MATERIAL AND METHODS

Homogeneously selected and surfaced sterilized (with 0.10% $HgCl_2$ solution) seeds of *Solanum melongena* were sown in solar energy trap, and in normal environmental (open field) conditions. Plants were grown in the polythene bags filled with the fixed amount of sandy loam soil. The growth pattern studies were made at an interval of 20 days from germination till maturity. Five phenotypically similar plants were picked up separately from both sides without damaging their root system. The plants were transported to the laboratory where they were washed thoroughly in running water to remove the adherent soil particles. General agricultural practices were adopted as a routine part of the study. The growth pattern studies were made on per plant basis (Kumar, 1981). Length, fresh weight, dry weight & water content of root, stem, leaf, flower and fruits were taken into consideration.

RESULTS AND DISCUSSION

Solar energy trap environment affected the growth pattern of different plant parts. The effect was observed at all the stages of the growth as compared to the normal.

The solar energy trap environment has inhibitory influence on the root growth of the plant (Table-1). The root length of the saplings was retarded to 83.91% of the control. It also reduced the fresh weight and dry weight of the root. Control condition proved to be helpful in 139.88% more fresh weight and 113.28% more dry weight of the roots. These results are confirmatory to the work of Smith *et al.* (1984) on cucumber plants. They observed that the shaded plants developed smaller root system with lesser dry matter content. The poor growth of the root system may be either because of the presence of high humid condition or the use of fungicides, which is usual practice in solar energy trap farming. For water requirements plants are not totally dependent on root system but can fulfill it partially from above ground plant parts too.

The microclimate of solar energy trap is favourable for root growth. The stem length is increased by 128.87%, its fresh weight by 150.77% and dry weight by 155.12% of the control. The water content of the stem reduced with the advancement of crop age. These results are confirmatory to the findings of Goudriaan and de Ruiter (1983), who observed the increased internodes length in the solar energy trap grown wheat plants. The dry matter content of the stem was reported 20% higher than the normal. Smith *et al.* (1984) working on tomatoes and cucumber observed more stem height and dry matter content in the plants grown under solar energy trap environment. Behera *et al.* (1990) observed twice more height and dry matter in the seedlings grown in a nursery, under solar energy trap

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environment. Better growth and more stem elongation inside the solar energy trap may be due to cumulative effect of the factors like soil and air temperature (Cure, 1985), light intensity (Smith *et al.,* 1984), carbon dioxide enrichment (Hopen and Ries, 1962; Hand, 1982; Kimball, 1986; Idso *et al.* 1987) and higher relative humidity (Mittra *et al.*, 1990).

The leaf number increased significantly (by 133.01%), their fresh weight by 136.06% and dry weight by 120.18% with the use of solar energy trap. Although the fresh and dry weight increased with the advancement of crop age in both the environments, yet the increase was significant under solar energy trap environment. The water content fluctuated throughout the life span of the crop. Similar findings were obtained by Singh *et al.* (1970) while working on Citrus seedlings, they reported that the leaves per seedlings were found to be increased under alkathene cover as compared to the normal. The dry matter of the leaves was 20% higher than the normally grown plants. Mittra *et al.* (1990) working on paddy, winter vegetables and flowers (*Zinnia* and *Cosmos)* reported that the increased leaf area promoted the photosynthetic efficiency of plants and as a result the dry matter accumulation was higher in tunnel grown plants than the normal ones.

The solar energy trap environment showed rapid increase in the flower number by 135.80%, although flowering was registered at 80-day stage in both the environments. The solar energy trap environment advanced the flowering by 4 days. The fresh and dry matter accumulation increased by 273.52% and 350% respectively, with the advancement of crop age under these conditions. The above results are confirmatory to the findings of Konyaeva and Korzinnikova (1983b). While working on tomatoes grown under polythene tunnels, they reported that these conditions increased the number of inflorescences of plants by 55.6% and the number of flowers by 64.9% when compared to the normal. Loeser (1986) observed that the flowering of *Heliopsis scabra* and *Aconitum nepellus* was advanced by 4 - 5 days with better quality.

The fruiting appeared at 100-day stage in both the environments. The solar energy trap environment increased the fruit number by 145.14%, their fresh weight by 130.63% and dry weight by 118.79% as compared to the normal environmental condition. The water content of the fruits reduced with the advancement of crop age in both the environments. The above results are confirmatory to the work of various workers. Hall and Besemer (1972) working on cucumber grown under plastic cover reported higher yield of quality fruits with a month earlier maturity. Cerda *et al.* (1983) reported that the yield and fruit weight of tomatoes grown under ethylvinyl acetate tunnel were highest. Konyaeva and Kozinnikova (1983a) observed that the tomatoes grown under polythene tunnels increased the fruit setting by 61.8%, fruits number by 31.0% and fruit weight by 21.0%, as compared to the normal. Peterson and Taber (1991) showed an early and higher tomato yield under polythene covers. Kumar *et al.*, (1997) reported 142.6% more fruits of *Vicia faba* under polythene house conditions. During the winter period carbon dioxide enrichment is especially valuable when poor light conditions limit growth and development (Rees & Hand, 1980). The production of more flowers and fruits may be due to favourable climate under polythene house (Sirjacobs, 1984). Raised greenhouse carbon dioxide concentration enhances photosynthesis and growth by increasing the rate of organic matter accumulation.

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Table 1 : Influence of solar energy trap environment on growth pattern of *Solanum melongena*.

Crop Age (Days)	Control plants				Solar Energy Trap raised plants			
	Length/no. (cm.)	Fresh weight (g Plant^{-1})	Dry weight (g Plant^{-1})	Water content (%)	Length (cm)	Fresh weight (g Plant^{-1})	Dry weight (g Plant^{-1})	Water content (%)
20(Stem)	8.00 ± 2.98	1.24 ± 0.47	0.07 ± 0.01	1671.00	8.78 ± 4.56	1.58 ± 0.04	0.12 ± 0.04	1216.66
(Root)	11.61 ± 2.65	1.09 ± 0.04	0.09 ± 0.05	1111.11	10.90 ± 2.98	0.98 ± 0.04	0.08 ± 0.05	1125.00
(Leaf)	3.40 ± 0.30	0.96 ± 0.13	0.19 ± 0.02	405.26	3.60 ± 0.57	0.86 ± 0.12	0.18 ± 0.03	377.78
(Flower)	-	-	-	-	-	-	-	-
40(Stem)	12.02 ± 3.67	6.21 ± 2.45	0.54 ± 0.09	1050.10	15.10 ± 1.34	8.78 ± 1.95	0.68 ± 0.16	1191.17
(Root)	14.62 ± 3.24	1.52 ± 0.44	0.14 ± 0.03	985.72	14.42 ± 1.93	1.46 ± 0.32	0.16 ± 0.04	812.50
(Leaf)	5.10 ± 0.90	2.15 ± 0.19	0.25 ± 0.02	760.00	5.80 ± 0.60	2.86 ± 0.27	0.29 ± 0.02	886.20
(Flower)	-	-	-	-	-	-	-	-
60(Stem)	22.30 ± 5.86	9.13 ± 1.27	0.89 ± 0.15	925.84	27.80 ± 6.09	19.87 ± +-3.56	1.87 ± 0.54	980.56
(Root)	17.34 ± 247	1.83 ± 0.83	0.17 ± 0.05	976.47	16.38 ± 3.62	1.78 ± 0.68	0.19 ± 0.04	836.83
(Leaf)	7.20 ± 1.00	3.90 ± 0.28	0.43 ± 0.03	806.87	8.20 ± 1.06	4.01 ± 0.36	0.49 ± 0.03	810.72
(Flower)	-	-	-	-	-	-	-	-
80(Stem)	31.02 ± 4.03	14.26 ± 3.04	1.61 ± 0.32	791.25	40.90 ± 3.94	27.55 ± 4.96	2.50 ± 0.73	1002.00
(Root)	18.68 ± 1.83	1.98 ± 0.45	0.20 ± 0.03	890.00	17.30 ± 1.94	1.88 ± 0.83	0.21 ± 0.05	795.23
(Leaf)	9.90 ± 1.10	6.40 ± 0.36	0.73 ± 0.06	776.71	11.70 ± 1.10	6.61 ± 0.52	0.75 ± 0.06	781.33
(Flower)	2.00 ± 0.15	0.04 ± 0.01	0.02 ± 0.001	100.00	3.00 ± 0.70	0.07 ± 0.02	0.03 ± 0.01	133.33
100(Stem)	36.03 ± 4.74	18.20 ± 1.05	2.30 ± 0.84	691.30	48.70 ± 4.82	30.06 ± 6.89	4.09 ± 1.84	781.24
(Root)	21.30 ± 3.87	2.36 ± 0.64	0.25 ± 0.07	840.00	18.78 ± 2.87	2.04 ± 0.75	0.26 ± 0.02	648.61
(Leaf)	14.30 ± 1.80	9.89 ± 1.21	1.12 ± 0.08	783.03	16.60 ± 1.60	10.10 ± 0.92	1.12 ± 0.08	801.78
(Flower)	4.00 ± 0.20	0.09 ± 0.01	0.03 ± 0.001	200.00	5.00 ± 0.94	0.13 ± 0.02	0.05 ± 0.015	160.00
(Fruit)	2.00 ± 0.20	35.17 ± 0.28	3.67 ± 0.09	858.31	3.90 ± 0.52	102.11 ± 3.98	3.64 ± 0.60	684.86
120(Stem)	47.50 ± 6.46	25.91 ± 4.93	3.51 ± 1.13	638.17	55.10 ± 7.74	44.05 ± 5.93	6.29 ± 1.46	699.50
(Root)	24.90 ± 4.95	5.20 ± 1.25	0.98 ± 0.56	430.61	21.72 ± 4.15	3.18 ± 0.83	0.49 ± 0.16	548.97
(Leaves)	18.70 ± 2.35	16.49 ± 1.56	1.92 ± 0.12	758.58	22.00 ± 2.32	20.24 ± 1.98	2.10 ± 0.28	863.80
(Flower)	8.10 ± 0.40	0.34+ ± 0.05	0.08 ± 0.02	325.00	11.00 ± 1.10	0.93 ± 0.12	0.28 ± 0.10	232.14
(Fruit)	4.20 ± 0.60	121.41 ± 0.98	11.01 ± 0.42	1102.72	6.20 ± 0.60	199.81 ± 4.72	13.01 ± 1.43	1435.81
Maturity (Stem)	48.11 ± 5.87	18.00 ± 4.73	4.39 ± 1.04	326.65	62.00 ± 6.82	28.24 ± 4.25	6.81 ± 1.85	314.68
(Root)	48.11 ± 5.87	18.00 ± 4.73	4.39 ± 1.0400	326.65	62.00 ± 6.82	28.24 ± 4.25	6.81 ± 1.85	314.68
(Leaves)	27.98 ± 4.91	7.40 ± 1.45	1.62 ± 0.26	356.79	23.48 ± 2.75	5.29 ± 0.78	1.43 ± 0.22	269.93
(Flower)	21.20 ± 2.10	20.99 ± 2.01	2.18 ± 0.18	862.84	28.20 ± 2.90	27.20 ± 2.65	2.91 ± 0.24	834.70
(Fruit)	-	-	-	-	-	-	-	-
	7.00 ± 1.40	266.61 ± 1.98	35.12 ± 1.35	659.14	10.16 ± 0.92	348.29 ± 6.83	41.72 ± 2.12	734.83

± Standard Deviation
Maturity Reached in: a. 158 days in the Control plants.
b. 148 days in the Solar Energy Trap Grown plants.

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Angiospermic Tree Species of Moradabad District of Uttar Pradesh

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An extensive survey has been conducted in Moradabad district for the study of total angiospermic trees grown and planted. The present paper enumerates 169 tree species belonging to 47 families which are spread out in all over the study area. *Ficus* species and *Psidium guajava* are frequently distributed whereas *Areca catechu, Commiphora mukul, Guazuma ulmifolia, Barringtonia acutangula, Semecarpus anacardium* are the rare tree species of Moradabad district.

Keywords : Angiosperms, Tree species, Moradabad.

INTRODUCTION

The district Moradabad is a part of Indo-Gangetic plain of North-West India in Uttar Pradesh. Biotic, mostly human, interferences such as cutting of trees for fuel or other purposes and cleaning of forests for cultivation, urbanization and industrialization have resulted in severe denudation the entire district. Today only the secondary forests, that too of insignificant economic value are present in discrete patches, showing practically no undergrowth during summer months. The area is rapidly facing industrialization and urbanization which changing the status of soil also. Paliwal and Singh (1982) took initiative to describe angiospermic diversity in Moradabad but after that taxonomical studies remained neglected. An attempt has been made to collect information about total number of angiospermic trees growing in the study area.

METHODOLOGY

Study Area : Moradabad is located at latitude 28° 50′ to 28° 83′ N and longitude 78° 47′ to 78° 78′ E and at a distance of 167 km (104 miles) from the national capital, New Delhi, on the banks of the Ramganga river (a tributary of the Ganges). District Moradabad cover an area of 3806.7 sq.km. and comprised of Moradabad Tehsil, Bilari Tehsil, Chandausi Tehsil, Sambhal Tehsil, Thakurdwara Tehsil and Kanth Tehsil and thirteen blocks. The area is characterized by periodic occurrence of hot summers, moderate rains and cold winters. All the elements of climate, *viz*; temperature, rainfall and relative humidity show well marked seasons. There are two transitional periods one between rainy and winter and the other between winter and summer season. Winter season is from November to February when the day length becomes short and night long and cool. Summer season starts from March and extends up to June. May and June are very hot when sun is scorching and the wind is strong and desiccating. Rainy season starts from the middle of June and in the first week of July and extended up to October. The maximum and minimum atmospheric temperatures are 44.2° C and 4° C respectively. The average rainfall varies between 800 to 1000 mm. The relative humidity is up to 90% in monsoon season and in drier part of the year it decreases to less than 20%.

An extensive survey has been conducted for two years (2007-2009) and during the period of study it has been put in mind that no place should be missed because every place is very useful to collect and record the new plant species. At every place I have gone and personally examine the trees and excursion were also made at least three times a week, otherwise 4 - 5 times in a month of the to see and watch the trees and to collect the information. Plants were identified with the help of flora (Hooker,1872-97; Duthie, 1903-1929; and Vardhana,2009) and herbarium (FRI. Dehradun and BSI. Allahabad). The survey area map enclosed for reference (Fig.1).

OBSERVATIONS

Plants play an important and vital role for the existence of life in the universe, but they are eradicating day by day to the urbanization from the original home. Here are given --- tree species those were studied in the survey.

MAGNOLIACEAE : *Magnolia grandiflora* L., *Michelia champaca* L.

ANNONACEAE : *Annona squamosa* L., *Polyalthia longifolia* Thw. Enum.

CAPPARIDACEAE : *Crateva religiosa* Hook.f.

FLACOURTIACEAE : *Casearia tomentosa* Roxb., *Flacourtia indica* (Burm.f.) Merr.

BIXACEAE : *Bixa orellana* L.

TAMARICACEAE : *Tamarix aphylla* (L.) Lanza in Boll., *T.dioica* Roxb., *T.troupii* Hele in Ind.

MALVACEAE : *Thespesia populnea* (L.) Sol.

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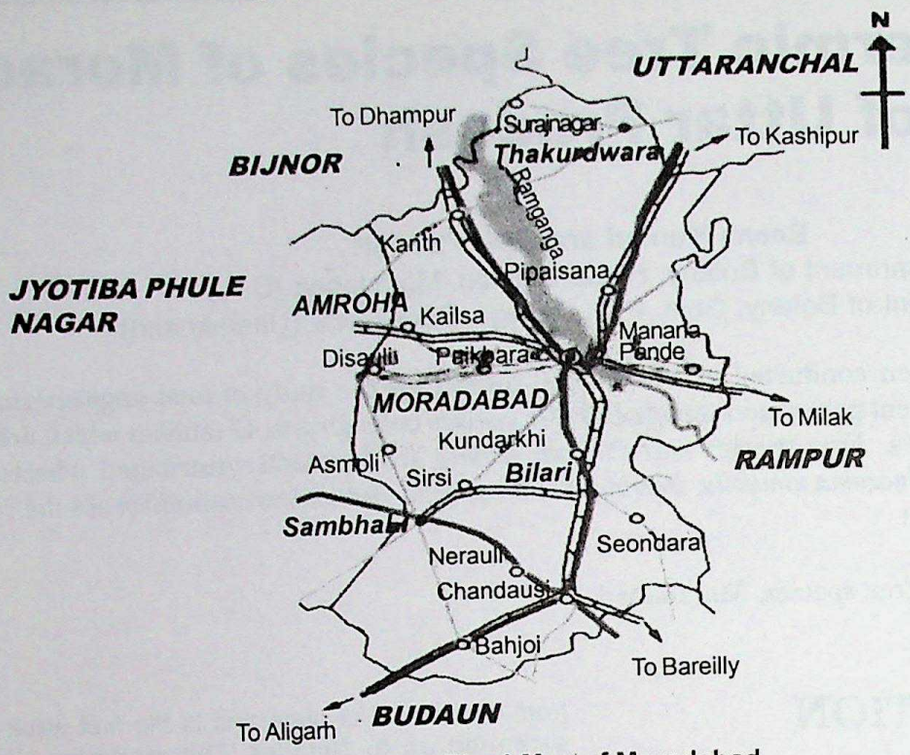

Fig. 1 Map of Moradabad

BOMBACACEAE : *Adansonia digitata* L., *Bombax ceiba* L.
STERCULIACEAE : *Guazuma ulmifolia* Lam., *Pterospermum acerifolium* (L.) Willd., *Stercuilia foetida* L.
TILIACEAE : *Grewia asiatica* L. *Grewia salvifolia* L.f.,
OXALIDACEAE : *Averrhoa carambola* L.
RUTACEAE : *Aegle marmelos* (L.)Correa., *Ailanthus excelsa* Roxb., *Citrus aurantifolia* (Christm.) Swingle., *C. limon* (L.)Burm.f., *C.maxima* (Burm.)Merr., *Feronia limonia*(L.) Swingle., *Murraya koenigii* (L.) Spreng., *M. paniculata* (L.)Jack.
SIMAROUBACEAE : *Ailanthus excelsa* Roxb.
OCHNACEAE : *Ochna obtusata* ssp.*gamble* (King ex Brandis) Kanis.
BURSERACEAE : *Boswellia serrata* Roxb. ex *Colebr., Commiphora mukul* Engl., *Garuga pinnata* Roxb.
MELIACEAE : *Azadirachta indica* A.Juss., *Chukrasia tabularis* A., *Melia azedarach* L., *Toona ciliata* Roem., *Swietenia mahogni* (L.) Jaca.
RHAMNACEAE : *Ziziphus mauritiana* Lam.
SAPINDACEAE : *Litchi chinensis* Sonner., *Sapindus trifoliatus* L., *Schleichera oleosa* (Lour.) Oken.
ANACARDIACEAE : *Lannea coromandelica* (Houtt)Merr., *Mangifera indica* L., *Semecarpus anacardium* L.f., *Spondias pinnata* (L.f.) Kuntz.
MORINGACEAE : *Moringa oleifera* Lam.
FABACEAE (PAPILIONACEAE) : *Butea monosperma* (Lam.)Taubert., *Dalbergia sissoo* Roxb., *Erythrina suberosa* Roxb., *E. variegata* L., *Pongamia pinnata* (L.)Pierre., *Sesbania grandiflora* Pers.
CAESALPINIACEAE : *Bauhinia purpurea* L., *B. racemosa* Lam., *B.variegata* L., *Brownea coccinea* Loefl ex Griseb., *Caesalpinia crista* L., *Cassia fistula* L., *C. javanica* L., *C.obtusifolia* L., *Delonix regia* (Boj.) Rafin., *Parkinsonia aculeata* L., *Pelthophorum pterocarpum* (DC.) Backer. exHeyne, *Saraca indica* L.,*Tamarindus indica* L.,
MIMOSACEAE : *Acacia auriculiformis* A.Cunn ex Benth., *A.arabica* Willd., *A.catechu* Willd., *A.nilotica* (L.)Del., *A.modesta* Wall., *Albizia lebbeck* (L) Benth., *A. odoratissima* (L.f.)Benth., *Leucaena leucocephorla* (Lam.) DeWit., *Pithecellobium dulce* [Roxb.] Benth., *Prosopis juliflora* (Sw.)DC., *Samanea saman* (Jacq.)Merr.
ROSACEAE : *Eriobotrya japonica* (Thumb.)Lindl., *Prunus persica* (L.)Stokes., *Pyrus communis* L., *P. sinensis* Hort.
COMBRETACEAE : *Anogeissus latifolia* Bedd., *Terminalia arjuna* (Roxb ex DC.)Wt.&Arn., *T.bellirica* (Geart.) Roxb., *T.tomentosa* Wt. & Arn.
MYRTACEAE : *Callistemon lanceolatus* DC., *Eucalyptus camaldulensi* Dehnh., *E. globulus* Labill., *E citriodoras* Hooker in Mitch., *E. panniculata* Sm.in Tls., *E. rudis* Endl.in Enum., *E. tereticornis* Sm.Bot., *E. rostrata* Schl.in Linnaea., *Melaleuca leucadendron* L., *Psidium guajava* L., *Syzygium cumini* (L.)Skeels in US Dept..
LECYTHIDACEAE : *Barringtonia acutangula* L.
LYTHRACEAE : *Lagerstroemia speciosa* L.
PUNICACEAE : *Punica granatum* L.
CARICACEAE : *Carica papaya* L.
RUBIACEAE : *Hamelia patens* Jacq., *Gardenia latifolia* Ait., *Mitragyna parvifolia* (Roxb.)Korth.
SAPOTACEAE : *Achras zapota* L., *Madhuca indica* Gmelin., *Manilkara hexandra* (Roxb.)Dubard., *Mimusops elengi* L.
EBENACEAE : *Diospyros melanoxylon* Roxb., *D. malabarica* (Ders.)*Kostel.*
OLEACEAE : *Nyctanthes arbor- tristis* L.
APOCYNACEAE : *Alstonia scholaris* (L.)Br., *Carissa carandas* L., *Plumeria rubra* L., *P.rubra* L.forma *acutifolia* Poir., *Thevetia peruviana* (Pers.)Merr., *Wrightia tincoria* R.Br.
ASCLEPIADACEAE : *Calotropis gigantia* (L)R.Br.
EHRETIACEAE : *Cordia dichotoma* Forsk., *C. sebestena* L. *Ehretia acuminata* R.Br., *E. laevis* Roxb.

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BIGNONIACEAE : *Fernandoa adenophylla* (Wall. ex G.Don)Van Steens., *Jacaranda mimosifolia* D.Don, *Kigelia pinnata* DC., *Millingtonia hortensis* L.f., *Oroxylum indicum* (L.)Benth ex Kurz., *Spathodea campanulata* Beauv., *Tabebuia pentaphylla* (L.) Hemsl., *Tecoma stans* (L.) H.B.&K., *Tecomella undulata* (Sm.) Seem.

VERBENACEAE : *Citharexylum spinosum* L., *Gmelina arborea* Roxb., *Premna mucronata* Roxb., *Tectona grandis* L.f., *Vitis negundo* L.

PROTEACEAE : *Grevillea robusta* A.Cunn.

EUPHORBIACEAE : *Emblica officinalis* Gaertn., *Euphorbia nerifolia* L., *E.roylena* L., *Jatropha curcas* L., *Putranjiva roxburghii* Wall., *Ricinus communis* L., *Sapium sebiferum* (Michx.)Roxb.

ULMACEAE : *Holoptelea integrifolia* Roxb.

MORACEAE : *Artocarpus gomezianus* ssp. *zeylanicus* Jarret., *A. heterophyllus* Lam, *Ficus benghalensis* L., *F. carica* L., *F. glomerata* Roxb., *F. krishnae* C., *F. racemosa* L., *F.rumphii* Bl. *F. religiosa* L., *F. virens* Ait., *Morus alba* L., *M. indica* L., *Streblus asper* Lour.

CASUARINACEAE : *Casuarina equisetifolia* J.R.&G.

SALICACEAE : *Populus alba* L., *Salix tetrasperma* Roxb.

ARECACEAE : *Areca catechu* L., *Borassus flabellifer* L., *Caryota urens* L., *Livistona chinensis* R.Br., *Phoenix sylvestris* (L.)Roxb., *P.dactylifera* L., *Roystonea regia* (Kunth.)O.F.

POACEAE : *Bambusa arundinacea* (Retz.) Willd., *B. vulgaris* Sch.ex Wend.

CONCLUSION

During the survey of Moradabad district a total of 169 tree species were recorded belongings to 47 families. Majority of the tree species are used as fuel, food, fibre, timber and other purposes. Eight species of *Ficus* and *Psidium guajava* are frequently distributed over the entire study area whereas *Areca catechu*, *Commiphora mukul*, *Guazuma ulmifolia*, *Barringtonia acutangula*, *Semecarpus anacardium* are the rare tree species of Moradabad district.

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A Study on the Flora of Kolanthamamalai Hill, Tirunelveli District, Tamil Nadu

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An attempt was made to study the medicinal wealth of Kolanthamamalai hill an offshoot of Kalakadu Mundanthurai Tiger Reserve of Southern Western Ghats in Tirunelveli district, South India during 2006. The team has recorded 148 species covering 119 genera and 53 families. This paper deals with medicinal aspects of these species in brief besides their diversity. The rural folk of the adjacent villages regularly collect medicinal plants from this area. The unsystematic and unorganized collection of medicinal plant for raw drug created heavy decline of medicinal plants population in their natural habitat. The collection of bark, root and tuber cause serious threat to the medicinal plant population.

Keywords : Biodiversity, Conservation, Medicinal flora.

INTRODUCTION

India possesses a great heritage of ancient systems of medicine such as Siddha, Ayurveda, Unani and Homeopathy. In addition to these traditional systems, there also exists a vast knowledge of tribal and folk medicine in India, which utilize around 7500 species of plants as medicinal. Some of the ethno botanically important species have also provided leads for production of many drugs. It is estimated that in India 90% of the prescriptions contain plant products (Husain & Akhtar 1983). Due to regular commercial exploitations like plantation programmers, mining and quarrying, urbanization, fragmentation of habitats and wild population occur at a rapid rate. For survival of endemic species and their populations more intensive management is needed, such as systematic survey, status assessment, habitat management, restoration and strategies.

The altitude of the hilly rugged terrain ranges from 150 to 300 m with the highest peak being Kolanthamamalai (Map: I). Geologically, the soil is reddish-black sandy loam intermingled with occasional granitoid rock. The Kolanthamamalai hill experience both south-west (July - September) and north-east (October - December) monsoons and occasional showers. Annual rainfall varies from 800-1200 mm. Temperature is (38°C) maximum during the months of April - June and (20°C) minimum during November - December.

MATERIAL AND METHODS

The natural barriers, habitat diversity, climate and moderate rainfall have resulted in the development of unique plant diversity. The Kolanthamamalai hill is one of the important forest reserves of Tirunelveli district, an offshoot of Kalakadu Mundanthurai Tiger Reserve of Southern Western Ghats. The study area is about 25km from Tirunelveli and can be reached by a motorable road. It lies between 77°10'- 77°40'E and 8°25' -8°53'N. The hill is surrounded by the revenue villages Cheranmahadevi on the north, Padamadai on the east, Kalakadu Mundanthurai Tiger Reserve on the south and Kalakadu on the west. KMTR is one of the richest phytogeographical regions of Tamil Nadu with about 2000 species of flowering plants. The physiography of this range differs much from the adjacent portions of Southern Western Ghats of Tamil Nadu state due to the varying altitude, ridges and their height and breadth.

Field visits were made during 2006 in the study area to survey and togather information on herbal medicines and to collect voucher specimens for further study. To confirm the identification of the species, Gamble's Flora of the Presidency of Madras (1935), Mathew (1983 -1988), Rennet (1987) and Flora of Tamil Nadu (1983, 1987 and 1989) were referred. The medicinal uses of plants were compiled with the help of earlier publication. (Chopra 1956; Useful plants of India 1986; Yoganarasiman 2002).

RESULTS

The vegetation of Kolanthamamalai hill is mixed dry deciduous type. Low land vegetation girdles the base of the mountain on the eastern and southern side of the hill. Along the foot hill vegetation is an open scrub jungle with occasional trees. Exotic species like *Prosopis juliflora, Acacia farnifera* are eco- dominated in eastern side of the hill. In southern side

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Table 1 : Medicinal flora of Kolanthamamalai hill and their uses.

S.No.	Name of the species	Habit	Vernacular Name	Medicinal uses
			DICOTYLEDONS	
	ACANTHACEAE			
1.	*Andrographis alata* (Vahl) Nees	Herb	Nila vembu	Whole plant, used orally in dysentery, cholera, diabetes, influenza, bronchitis, itches and piles.
2.	*Andrographis echioides* Nees	Herb	Gopuram thangi	Whole plant used in dysentery, influenza, bronchitis, itches and piles.
3.	*Asystasia gangetica* T.	Herb	Pasathy keerai	Plant juice administered orally to children suffering from swellings and rheumatism.
4.	*Barleria cuspidata* Heyne ex Nees	Shrub	Chunnambu korandi	Leaves and roots used orally for cough and inflammation.
5.	*Barleria longiflora* L.f.	Herb	-	Antidote for poison of plant origin.
6.	*Barleria nitida* Nees	Shrub	-	Leaves and roots used orally for cough and inflammation.
7.	*Barleria prionitis* L.	Shrub	Semmulli	Root paste applied to boils and glandular swellings.
8.	*Ruellia prostrata* Poir.	Herb	Vedikkai	Used in ear troubles
	AMARANTACEAE			
9.	*Alternanthera sessilis* R.Br.	Herb	Ponnankanni	Whole plant used orally for night blindness, fever and in leprosy.
10.	*Celosia argentea* L.	Herb	Pannaikeerai	Seeds used orally in diarrhoea, eye troubles and sores in mouth.
11.	*Psilotrichum elliotii* Baker & C.B. Clarke.	Herb	-	Whole plant eye sight with Cow's milk.
	APOCYNACEAE			
12.	*Carissa carandas* L.	Shrub	Kalakkay	Fruits used orally in indigestion, vomiting, hypertension.
13.	*Wrightia tinctoria* (Roxb.) R.Br.	Tree	Vetpalai	Leaf, latex used orally in psoriasis, eczema and in dysentery.
	ARISTOLOCHIACEAE			
14.	*Aristolochia indica* L.	Climber	Karudakkodi	Roots antitode. Leaf juice used orally in cough.
	ASCLEPIADACEAE			
15.	*Calotropis gigantea* R.Br.	Shrub	Erukku	Root bark used in leprosy, convulsions, joint swelling.
16.	*Hemidesmus indicus* (L.) R.Br.	Climber	Nannari	Dried roots used orally as demulcent, diaphoretic and diuretic.
17.	*Pergularia daemia* (Forssk.)Choiv.	Climber	Velipparuthi	Leaf decoction used orally in asthma, infantile diarrhoea and rheumatic swellings
18.	*Sarcostemma acidium* (Roxb.) Voigt	Climber	Kodikkalli	Root infusion given to persons bitten by dogs.
19.	*Tylophora indica* (Burm.f.) Merr.	Climber	Nayppalai	Roots used orally in asthma, bronchitis, whooping cough, dysentery and diarrhoea.
20.	*Wattaaka volubilis* (L.f.) Stapfd.	Climber	Kurincha	Leaves used in abscesses.
	ASTERACEAE			
21.	*Ageratum conyzoides* L.	Shrub	Pumpillu	Whole plant used orally as nervine tonic, Decoction or infusion used orally in diarrhoea, dysentery, colic with flatulence and other gastro - intestinal ailments.
22.	*Bidens pilosa* L.	Herb	-	Herb used externally in leprosy and other skin troubles, tumours.
23.	*Eclipta prsotrata* L..	Herb	Karisalai	Whole plant used orally in hepatic and spleen enlargements and externally in skin troubles.
24.	*Emilia sonchifolia* DC.	Herb	Neichitti poondu	Leaf juices used externally in eye sores and night blindness.
25.	*Notonia grandiflora* DC.	Shrub	Thelkadi thalai.	Leaf juices apply on scorpion bite.
26.	*Vernonia cinerea* (L.) Less.	Herb	Neychitti	Herb infusion makes combination with quinine in malaria. Leaf decoction given orally in diarrhoea and stomachache, cough and colic.
27.	*Vicoa indica* DC.	Herb	Kattusuriagandhi	Plant juices given orally for antifertility.
28.	*Xanthium indicum* Roxb.	Shrub	Maruloomathai	Root extract applied upon ulcers, boils and abscesses. Leaves used in scrofula and herpes. Fruits used orally in urino-genital disorders.

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	ANACARDIACEAE			
29.	*Lannea coromandelica* (Houtt.)Merr.	Tree	Othiyam	Barks apply externally upon wounds, inflammation, and in ulcer.
	AIZOACEAE			
30.	*Mollugo cerviana* Ser.	Herb	Parpadapal	Plant juices used orally in promoting lochial discharges and in gonorrhoea
31.	*Mollugo nudicaulis* Lam.	Herb	Parpadagam	Plant juices used orally in whooping cough. Leaves applied to boils for suppuration.
32.	*Mollugo pentaphylla* L.	Herb	Turapoondu	Plant juices apply upon in skin diseases and earache.
33.	*Trianthema portulacastrum* L.	Herb	Vellai charanai	Roots orally used in asthma, amenorrhea and obstruction in liver.
	BORAGINACEAE			
34.	*Carmona retusa* (Vahl.) Masam.	Shrub	Kuvitchipoondu	Fresh root used as an antidote to vegetable poisoning. Leaf decoction used orally for cough and stomach troubles.
	CAESALPINIACEAE			
35.	*Cassia absus* L.	Herb	Mulaipalvidai	Seeds externally used in opthalmia and skin troubles,
36.	*Cassia auriculata* L.	Shrub	Avaram	Leaves and fruits used orally in eye troubles and diabetes, Roots employed externally in skin troubles.
37.	*Cassia fistula* L.	Tree	Sarakonnai	Dried fruit pulp used externally as purgative, laxative in habitual constipation.
38.	*Cassia tora* L	Herb	Tagarai	Leaves purgative, used externally in ringworm and other skin troubles.
	CAPPARACEAE			
39.	*Cadaba trifoliata* (Roxb.) W. & A.	Tree	Vezhi	Roots and leaves crushed apply upon rheumatism.
40.	*Capparis sepiaria* L.	Shrub	Karunjoorai	Root barks apply upon skin troubles.
41.	*Cleome aspera* Koenig	Herb	Vallai	Seeds useful in toothache
42.	*Cleome viscosa* L.	Herb	Naikkadugu	Seeds used orally as anthelimintic.
	CARYOPHYLLACEAE			
43.	*Polycarpaea corymbosa* (L.) Lam.	Herb	Nilasadachi	Leaves pounded and applied as poultice, warm or cold, over boils and Inflammatory swellings.
	CELASTRACEAE			
44.	*Reissantia indica* (Willd.) Halle	Climber	Murasangodi	Root bark used orally for respiratory troubles.
	COMBRETACEAE			
45.	*Combretum ovalifolium* Roxb.	Climber	Kodi Maruthu	Leaves used orally as febrifuge.
	CONVOLVULACEAE			
46.	*Evolvulus alsinoides* L.	Herb	Vishnu karandai	Plants tonic, febrifuge and vermifuge, used with coconut oil for promoting hair growth.
47.	*Ipomoea obscura* (L.) Ker Gawler	Climber	Siruthazhi	Leaves application in pathogenic infection.
48.	*Ipomoea pes-tigridis* L.	Climber	Pulichovadi	Leaves as poultice upon boils, sores, pimples and carbuncles.
49.	*Merremia tridentata* (L.) Hall.f.	Climber	Mudiyar koonthal	Whole plant used externally in rheumatism, hemiplegia, piles and urinary disorders.
50.	*Rivea hypocrateriformis* (Desr.) Choisy	Shrub	Musuttai	Roots given after parturition.
	CUCURBITACEAE			
51.	*Corallocarpus epigaeus* (Rottl. & Willd.) C.B.Clarke.	Climber	Kollankovai	Root used externally in rheumatism.
52.	*Melothria madraspatana* Cogn.	Climber	Musumusukkai	Tender shoots and leaves prescribed orally for vertigo, biliousness. Roots masticated for relief from tooth-ache.
	ERYTHROXYLACEAE			
53.	*Erythroxylon monogynum* Roxb	Tree	Chempulichan	Infusion of root and bark stomachic, diaphoretic and diuretic, useful in dyspepsia and fever.
	EUPHORBIACEAE			
54.	*Phyllanthus emblica* L.	Tree	Nelli	Fruits orally as diuretic and laxative.
55.	*Euphorbia antiquorum* L.	Tree	Sadura -kalli	Whole plant purgative and digestive, decoction given in gout, Juice used in nervine troubles and dropsy.
56.	*Euphorbia dracunculoides* Sam.	Herb	Palperukki	Capsule used externally to remove warts,

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57.	*Euphorbia tortilis* Rottl. ex Ainsile,	Tree	Tirukalli	Latex used externally to remove warts, also applied in neuralgia and tooth-ache.
58.	*Phyllanthus amarus* L.	Herb	Keelanelli	Plant used orally in stomach disorders jaundice and diseases of the urino - genital system.
59.	*Phyllanthus maderaspatensis* L.	Herb	Melanelli	Leaves infusion given in headache
60.	*Phyllanthus reticulatus* Poir.	Shrub	Karumpoola	Bark used externally in rheumatism and venereal diseases.
	PAPILIONACEAE			
61.	*Abrus fruticulosus* Walich . ex W. & A	Climber	Vellai kuntri	Seeds used orally in defection of nervous systems, sciatica, stiffness of shoulder joints and paralysis.
62.	*Abrus precatorius* L.	Climber	Kundrimani	Seeds used orally in diseases of nervous system and in leucoderma.
63.	*Alysicarpus monilifer* (L.) DC.	Herb	Kurthikuzhai	Whole plant used in tooth ache.
64.	*Alysicarpus vaginalis* (L.) DC.	Herb	Kuranthiadakki	Plant used orally in uterine bleeding.
65.	*Atylosia scarabaeoides* (L.) Benth.	Climber	-	Root used externally in rheumatism.
66.	*Crotalaria verrucosa* L.	Herb	Kilukiluppai.	Crushed leaves apply on scabies and impetigo.
67.	*Desmodium triflorum* DC.	Herb	Sirupulladi	Leaves used orally in dysentery, diarrhoea and convulsions
68.	*Dolichos trilobus* L.	Herb	Panipeyar	Root juices apply upon ophthalmic and skin diseases.
69.	*Indigofera aspalathoides* Vahl ex DC.	Shrub	Sivanar vembu	Plant decoctions apply on cancerous affections and leprosy.
70.	*Indigofera caerulea* Roxb.	Shrub	Vellaiavuri	Root used orally in urinary complaints and hepatitis.
71.	*Indigofera tinctoria* L.	Shrub	Nili	Leaf extract used orally in epilepsy and others nervous disorders.
72.	*Pongamia pinnata* (L.) Pierre	Tree	Pungam	Root, bark, seed used externally in skin diseases.
73.	*Tephrosia purpurea* (L.) Pers.	Shrub	Kolingi	Whole plant used orally in bronchitis and bilious febrile attack.
74.	*Tephrosia villosa* (L.) Pers.	Shrub	Punaikkozhunji	Leaf juice given in dropsy.
75.	*Zornia diphylla* (L.) Pers	Herb	Porikarappan thazhai	Plants used orally in dysentery.
	LAMIACEAE			
76.	*Anisochilus carnosus* (L.f.) Wall.	Herb	Poochanthira pattai	Plant juice given orally in urticaria associated with liver disorders.
77.	*Anisomeles malabarica* R.Br.	Shrub	Perumthumbai	Leaves used orally in dyspepsia and fever.
78.	*Ocimum basilicum* L.	Herb	Naithulasi	Leaf used orally in digestive disorders and fever.
79.	*Ocimum tenuiflorum* L..	Herb	Thulasi	Leaves used in bronchitis, digestive disorders, cough and in fever.
80.	*Orthosiphon thymiflorus* (Roth) Sleensen	Herb	Akkiramkolli	Plant decoction used orally in diabetes and in urinary tract infection.
	LOGANIACEAE			
81.	*Strychnos potatorum* L.f.	Tree	Thettrankottai	Seeds used orally in diarrhoea, diabetes and gonorrhoea,
	LORANTHACEAE			
82.	*Dendrophthoe falcata* (L.f) Etting	Shrub	Pulluruvi	Bark used orally in menstrual troubles and asthma.
	LYTHRACEAE			
83.	*Ammannia baccifera* L.	Herb	Nirumel neruppu	Whole plant used externally in abscess and in polyuria.
	MALVACEAE			
84.	*Hibiscus ovalifolius* (Forsk.) Vahl	Shrub	Kuruvitchi	Plants used as febrifuge.
85.	*Pavonia odorata* Willd	Herb	Peramutti	Root used orally in dysentery and intestinal haemorrhage.
86.	*Sida acuta* Burm.f.	Herb	Arivalmooku patchilai	Roots used orally in nervous and urinary disorders and bowel complaints.
87.	*Sida cordifolia* L.	Herb	Sitramuttai	Root used orally in fevers and urinary complaints and in arthritis swellings.
	MELIACEAE			
88.	*Azdirachta indica* A.Juss	Tree	Vembu	Bark used externally in skin troubles. Leaves applied to boils in the form of poultice; decoction given for ulcers and eczema.
89.	*Toona ciliata* Roemer	Tree	Santhana vembu	Bark used in chronic dysentery of infants; used externally for ulcers

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	MENISPERMACEAE			
90.	*Cissampelos pariera* L.	Climber	Ponmusattai	Root used orally in dyspepsia, dropsy and urinary troubles.
91.	*Cocculus hirsutus* (L.) Diels	Climber	Kattukodi	Leaf juice used externally in eczema and impetigo.
92.	*Tinospora cordifolia* (Willd.) Hook. f. & Th.	Climber	Chinthil	Stem used orally in general debility, dyspepsia, fevers and urinary diseases.
	MIMOSACEAE			
93.	*Acacia nilotica* (L.) Dal.	Tree	Karuvelum	Decoction of bark used as gargle and pods in urino-genital diseases.
94.	*Acacia planifrons* W.& A.	Tree	Kodaivelam	Bark used orally in bronchitis and biliousness.
95.	*Albizia amara* Boiv.	Tree	Uchil	Root used orally in cough.
96.	*Dichrostachys cinerea* (L.) W. & A.	Tree	Vidathalai	Roots used externally in rheumatism.
97.	*Mimosa pudica* L.	Herb	Tottalsurunki	Decoction of root used orally in fever. Leaf juice used in dressings for sinus and also for sores and piles.
	MORACEAE			
98.	*Ficus amplissima* Smith.	Tree	Kalaththi	Bark used externally in ulcers and venereal diseases.
99.	*Ficus arnottiana* Miq.	Tree	Kal arasu	Leaves and bark used externally in cutaneous infections.
100.	*Ficus bengalensis* L.	Tree	Alamaram	Latex applied in rheumatism. Infusion of bark used in diarrhoea, dysentery and diabetes.
101.	*Ficus hispida* L.f.	Tree	Peyatthi	Bark used externally in skin diseases and ulcers.
102.	*Ficus racemosa* L.	Tree	Atthi	Bark, figs used orally in constipation, anemia and in dysentery.
103.	*Streblus asper* Lour.	Tree	Piray	Bark used orally in fevers, diarrhea, dysentery.
	NYCTAGINACEAE			
104.	*Boerhavia diffusa* L.	Herb	Mookarattai	Roots used orally in asthma.
	OLEACEAE			
105.	*Jasminum angustifolium* Vahl.	Climber	Kattu mullai	Root paste applied in ringworm.
	OXALIDACEAE			
106.	*Biophytum sensitivum* DC.	Herb	Jalapushpam	Plants used orally in disuria.
	PASSIFLORACEAE			
107.	*Passiflora foetida* L.	Climber	Jimmikkipoo	Fruits used in asthma and biliousness.
	PEDALIACEAE			
108.	*Sesamum indicum* L.	Herb	Ellu	Seed paste applied to piles. Fresh leaves used externally in ophthalmic and cutaneous complaints.
	PLUMBAGINACEAE			
109.	*Plumbago indica* L.	Shrub	Kodiveri	Roots used orally in leucoderma, syphilis and leprosy.
	POLYGALACEAE			
110.	*Polygala javana* DC.	Herb	Periyanangai	Whole plant used orally as antivenom.
	RHAMNACEAE			
111.	*Ventilago madraspatana* Gaertn	Climber	Vempadam	Root - bark used orally in debility and fevers.
112.	*Zizyphus mauritiana* Lam.	Shrub	Elandai	Whole plant used orally in diarrhea, dysentery and skin diseases.
113.	*Zizyphus oenophlia* (L.) Mill.	Climber	Soorai	Fruit used orally in fever and retention of urine.
114.	*Zizyphus xylopyrus* (Retz.) Willd.	Tree	Kottei	Root bark and fruit used orally in bronchial asthma, diarrhoea.
	RUBIACEAE			
115.	*Canthium parviflorum* Lam.	Tree	Karai	Root used orally in the disorders of throat.
116.	*Hedyotis puberula* R. Br.	Herb	Impooral	Leaves and roots given orally in asthma and bronchitis.
117.	*Ixora pavetta* Andr.	Tree	Sulandu	Leaf, stem and bark used externally in fever and headache.
118.	*Morinda pubescens* Smith	Tree	Manjanetti	Root, leaf and fruit used in eczema, fever and ulcers.
119.	*Spermacoce hispida* L.	Herb	Nattaichoori	Root used orally in dysentery
	RUTACEAE			
120.	*Toddalia asiatica* Lam.	Shrub	Milakaranai	Root bark used orally in diarrhoea and debility during convalesce.
	SAPINDACEAE			
121.	*Cardiospermum halicacabum* L.	Climber	Mudakkattankeerai	Root used orally in rheumatism and nervous diseases.

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SCROPHULARIACEAE			
122. *Lindernia crustacea* (L.) Mnell.	Herb	-	Used orally in bilious affections dysentery.
123. *Sopubia delphinifolia* Don	Herb	-	Plant juices apply in sores of feet.
SOLANACEAE			
124. *Datura metel* L.	Shrub	Oomaththai	Leaf juice applies in wounds, ulcers and skin diseases.
125. *Solanum surattense* Burm.f.	Herb	Kandan Kattiri	Berry used orally in cough, tuberculosis.
STERCULIACEAE			
126. *Helicteres isora* L.	Shrub	Valampiri	Fruits used externally in intestinal complaints.
TILIACEAE			
127. *Corchorus aestuans* L.	Herb	Vannan poondu	Leaves used orally in stomach disorders.
128. *Grewia villosa* Willd.	Shrub	Kodiunnu	Roots used orally in diarrhoea.
129. *Triumfetta rhomboidea* Jacq.	Herb	Adaiotti	Roots used in dysentery. Powdered roots given in intestinal ulcers. Leaves and flowers used externally in leprosy.
ULMACEAE			
130. *Celtis philippensis* Blanco	Tree	Vakkanai	Stem bark used orally in easy delivery for women.
VERBENACEAE			
131. *Lantana camera* L.	Shrub	Unni	Root used orally in tetanus, rheumatism and malaria.
132. *Phyla nodiflora* (L.) Greene	Herb	Poduthazhai	Whole plant is used orally in dysentery and leucorrhoea.
133. *Premna latifolia* Roxb.	Shrub	Munnai	Leaves used orally in diuretic.
VITACEAE			
134. *Cyphostemma setosum* (Roxb.) Alston	Climber	-	Leaves applied to ulcers for promoting suppuration.
135. *Cissus quadrangularis* L.	Climber	Puli Perandai	Juice prescribed orally in scurvy. Powdered roots considered specific for fractures.

MONOCOTYLEDONS

AGAVACEAE			
136. *Agave angustifolia* Haw.	Shrub	Kathalai	Leaf and root used orally to discharge the retention of urine.
AMARYLIDACEAE			
137. *Curculigo orchioides* Gaertn.	Herb	Nilappanaikizhangu	Tuberous roots used orally in diarrhoea, piles, jaundice and asthma.
COMMELINACEAE			
138. *Commelina benghalensis* L.	Herb	Kanavalai	Herb used externally in leprosy.
139. *Anischophacolus axillaris* (L.) Rookkan	Herb	Valukaipul	Plant used externally in leprosy.
CYPERACEAE			
140. *Cyperus rotundus* L.	Herb	Korikizhangu	Tubers used orally in fevers and bowel complaints.
141. *Kyllinga brevifolia* Rottb.	Herb	-	Leaves used orally in diarrhoea.
142. *Scleria lithosperma* (L.) Sw.	Herb	-	Decoction of roots given orally after parturition.
DIOSCOREACEAE			
143. *Dioscorea bulbifera* L.	Climber	Pannivalli.	Tubers applied to ulcers, piles and orally in dysentery.
144. *Dioscorea wallichi* Hook.f.	Climber	Mulvalli	Tubers in powder from applied to ulcers, piles and orally in dysentery
LILIACEAE			
145. *Asparagus racemosus* Willd.	Climber	Thanneervittan kizhangu	Fresh root juice in mixed honey given orally in dyspepsia
146. *Gloriosa superba* L.	Climber	Kalappai kizhangu	Tubers used externally in snake bite and scorpion bite poisoning and in skin diseases.
POACEAE			
147. *Aristida adscensionis* L.	Herb	Oosipul	Leaf used orally in diuretic.
148. *Cymbobogon flexuosus* (Neesex Steud) Wats.	Herb	Sukkunari	Leaf used orally in carminative.

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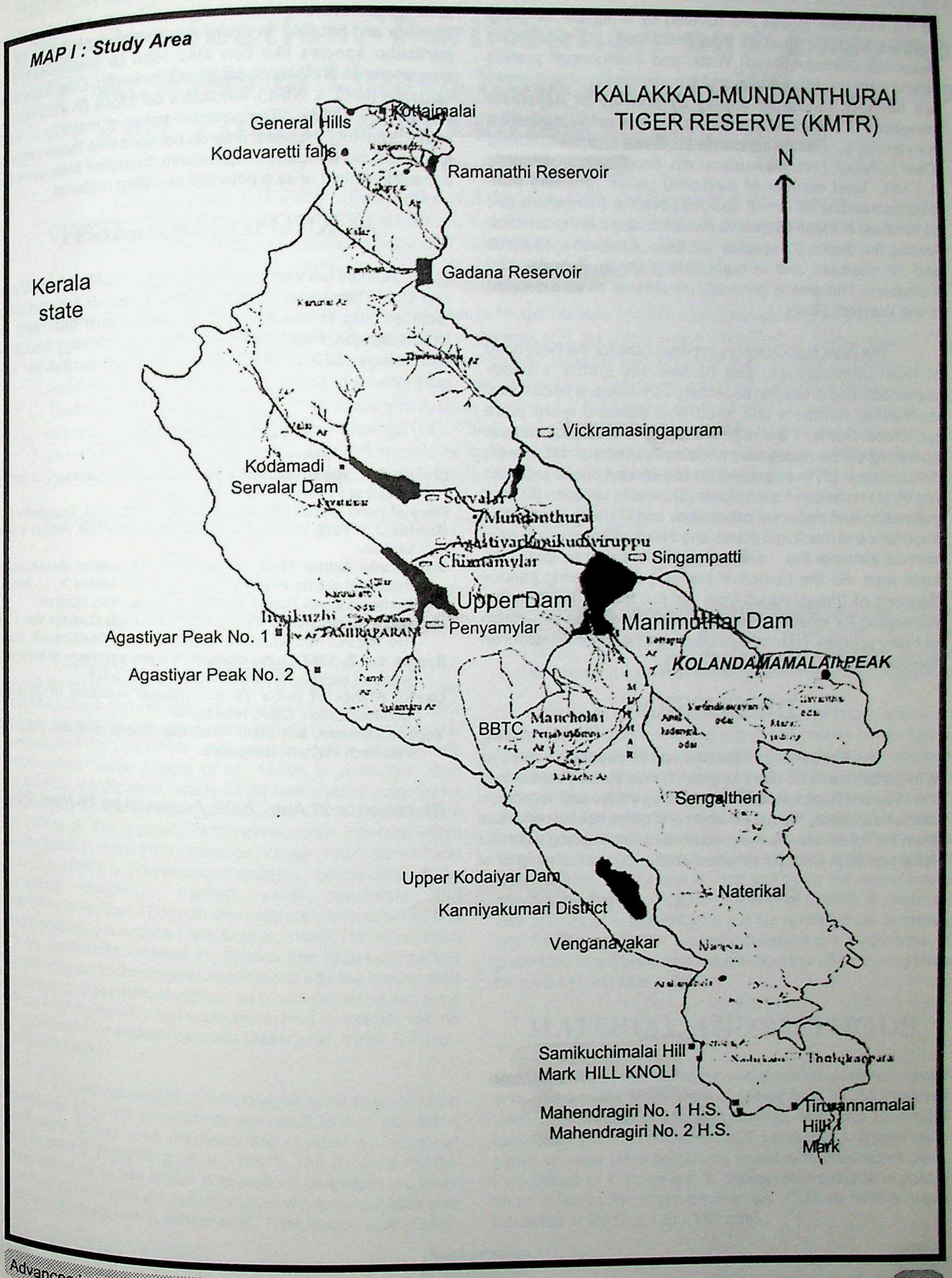
MAP I : Study Area



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of the mountain slopes are covered by a number of grass species such as *Aristida adscensionis* L., *Cymbobogon flexuosus* (Neesex Steud) Wats. and *Andropogon pumilis* Roxb. The low lying areas and the seasonal water courses are densely vegetated mostly by species of *Asystasia gangetica* T., *Wattaaka volubilis* (L.f.) Stapfd., *Ageratum conyzoides* L., *Cassia auriculata* L., *Rivea hypocrateriformis* (Desr.) Choisy, *Toddalia asiatica* Lam. and *Zizyphus oenophlia* (L.) Mill. Total number of medicinal plants recorded from Kolantamamalai hill is 148 species covering 119 genera and 53 families, of these 46 genera are dicots and 7 are monocots. Among the dicots, 23 species are trees, 41 shrubs, 43 herbs and 28 climbers and in monocots 1 shrub, 7 herbs and 4 climbers. The area is generally considered as an extension of the Western Ghats.

The plant resources are important and for the lively hood of local communities. The biodiversity status is under unprecedented pressure, especially from humans whose ever increasing numbers will need and demand more plant resources. Some of the negative (biotic and abiotic) impacts operating on the ecosystem of Kolanthamamalai hill are (1) deforestation (2) over grazing (3) soil erosion due to increase run off (4) invasion of alien plants (5) climatic variation (6) poor pollination and dispersal capabilities and (7) flash floods. The importance of medicinal plants and their traditional use to treat various ailments like are well known to many people in the local area. So the medicinal plants are also facing various degrees of threat. Apart from habitat loss, unsustainable harvesting for local markets also endangered the population of many species. Natural disasters also have some impact on the population.

DISCUSSION

The Kolanthamamalai hills and its vegetation serve as a migratory route for many tropical, humid floristic elements to the Western Ghats. Apart from the unique flora and vegetation of the mountains, the run off water and small streams originate from the forest are the main sources of replenishing water for local ponds in Cheranmahadevi area. However, a number of anthropogenic factors have adverse environmental impact on the flora and habitats. In many cases over exploitation of a particular species like can also lead to the incidental disappearance of other non-targeted species eg. *Corallocarpus epigaeus* (Rottl. & Willd.), *Tinospora cordifolia* (Willd.) Hook. f. & Th. C.B. Clarke. The potential value of majority of wild plants is not fully exposed. If we do not conserve these natural resources, we will lose the opportunity to exploit their uses as a medicinal plant or as a potential breeding material.

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Production, Purification and Enzyme Activity of Lipase from *Aspergillus oryzae* and *Trichoderma viride*

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Lipases are placed after proteases and carbohydrases in world enzyme market and share about 5% of enzyme market. They occur in plants, animals and microorganisms and are accordingly classified as plant, animal and microbial lipases. Wherever they exist, they function to catalyze hydrolysis of triglycerides to glycerol and fatty acid. Fungi are the potent source for the lipase production. The pure culture of *Aspergillus oryzae* and *Trichoderma viride* shows maximum lipase production in 96 and 72–96 hours respectively. Lipase Assay was done by using para Nitrophenyl Palmitate (pNPP). The lipase activity was determined from the crude enzyme as well as 35%, 60% and from dialyzed sample of lipase. Enzyme activity, protein content as well as total lipase activity was observed more in case of *Aspergillus oryzae* rather than *Trichoderma viride.*

Keywords : Lipases, Enzyme activity, *Aspergillus oryzae, Trichoderma viride.*

INTRODUCTION

Lipases (EC 3.1.1.3) are a class of hydrolases that are primarily responsible for the hydrolysis of acylglycerides. They are ubiquitous and indispensable for the bioconversion of lipids (triacyglycerol) in nature showing activity on a great variety of substrates (Brockerhoff and Jensen, 1974; Macrae and Hammond, 1985; Jaeger *et al.,* 1999). In addition to their biological significance, lipases hold tremendous potential for exploitation in biotechnology. They posses the unique feature of acting at the aqueous and non-aqueous interface which distinguishes them from esterases (Verger, 1997; Schmidt and Verger, 1998). In non-aqueous systems, lipases catalyse the reverse reaction, namely ester synthesis and transesterification. They can also catalyse stereoselective and regioselective reactions (Jaeger *et al.,* 1999). Therefore, there is an increasing interest for lipases and lipase-producting strains for biotechnological applications and the biochemical properties of lipolytic enzymes. as lipase-catalysed reactions show high selectivity and occur under mild conditions, with no requirement for added cofactors (Jager *et al.,* 1999; Schmidt-Dannert 1999).

The development of technologies using lipases for the synthesis of novel compounds will result in their expansion into new areas and increase the number of industrial applications (Bjorkling *et al.*, 1991). The demand for the production of highly active preparations of lipolytic enzymes has led to research on lipase producing microorganisms and on culture strategies (Lechner *et al.,* 1988; Suzuki *et al.*, 1988). Different studies have been published on the selection of lipase producers (Yamuguchi *et al.,* 1973; Kordel *et al.,* 1991; Lee and Rhee, 1993; Taipa *et al.,* 1994), but less information is available on the fermentation process for lipase production.

Lipases from fungi and bacteria serve important role in human practices as ancient as yogurt and cheese fermentation. Fungi are preferable lipase sources because fungal enzymes are usually excreted extracellularly, facilitating extraction from fermentation media. In the present study, a protocol has been developed for extraction of lipases from *Aspergillus oryzae* and *Trichoderma viride. Aspergillus oryzae* is a filamentous fungi that produces various useful enzymes and is useful in food industry. The fungus is used in *Koji* for the manufacture of sake, miso and soy sauce (Toida *et al.*, 1998). It produces less amount of lipase enzyme but these contribute in quality and function of miso. Lipases catalyze the hydrolysis of glycerides of soybean and the synthesis of ester during miso formation (Toida *et al.*, 1998).

MATERIAL AND METHODS

Microorganism and culture conditions : *Aspergillus oryzae* and *Trichoderma viride* were obtained from NCIM (National Center for Industrial Microorganism), National Chemical Laboratory, Pune. The colonies of *T. viride* and *A. oryzae* were grown on agar plate containing rhodamine (fluroscent dye). Pure culture of *T. viride* and *A. oryzae* were cultured in potato broth medium for mass production. Culture broths were incubated at 28ºC at 120 - 130 rpm.

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Lipase Assay using para Nitrophenyl Palmitate (pNPP) : Para Nitrophenyl esters of various chain length fatty acids are generally used as substrate and release of p- Nitrophenyl is measured spectrophotometrically at 410 nm. However, short chain esters are water-soluble and therefore their hydrolysis provides a measure of esterase. rather than lipase activity. In this investigation p-Nitrophenyl palmitate is used to measure lipase activity.

Preparation of substrate solution (emulsion): The substrate solution was prepared by adding solution A (Solution A: 40 mg of p-Nitrophenyl palmitate in 12 ml of Propanol) to the 9.5 ml of solution B (Solution B: 0.1 ml of Tween 80 and 0.4 ml of Triton x 100 in 90 ml of distilled water) dropwise with intense stirring. The emulsion obtained remained stable for at least 2 hrs. (Winkleker and Stuckmann, 1979).

Purification of Crude enzyme : All operations were carried out at a temperature of 4°C. On the 4th day of *Aspergillus* culture and on the 2nd day of *Trichoderma* culture, the culture broth were filtered with cotton gauze to remove the mycellial growth and centrifuge in cooling centrifuge at 10,000 rpm for 10 minutes at 4°C. The supernatant was brought to saturation concentration of 35% with ammonium persulphate. Solution was continuously stirred on magnetic stirrer for about 4 hours at 4°C followed by centrifuged at 10,000 rpm. The supernatant was collected and the obtained precipitation was dissolved in distilled water. The assay mixture was consisted of 900 µl of substrate and 100 µl of crude enzyme and incubated at 37°C for 30 minutes and reaction was stopped by adding of 2 ml of acetone followed by the addition of 1p nitrophenyl; the 1p nitrophenyl released was measured at 410 nm spectrophotometerically. The optical density of test was extrapolated on standard graph of pNitrophenol and concentration was estimated. Now, to get 60% precipitation, for 100 ml of crude enzyme 15.3 g ammonium persulphate is added. Then it was kept still for overnight at 4°C. After that centrifugation was carried at 10,000 rpm in at 4°C. The precipitate obtained was dissolved in distilled water and lipase assay was carried out. Dissolved precipitate was further kept for dialysis against distilled water for 4 hours at 4°C.

RESULTS AND DISCUSSION

Lipase production by *Aspergillus oryzae* and *Trichoderma viride* was confirmed on Rhodamine B agar plates containing olive oil. The colonies showed pink-orange coloured fluorescence when exposed to U.V light. This is due to the formation of complex between cationic Rhodamine B and the fatty acid ion.

Rate of growth and enzyme production by micro-organism is quite important in understanding their fermentation pattern. The rate of production of lipase enzyme by *Trichoderma viride* in potato broth culture with respect to 48 to 72 hours shown in Fig 1. In *Aspergillus oryzae* the lipase production was observed maximum after the incubation period of 96 hours (Fig.2). Kashmiri *et al.,* (2006) reported enzyme production from *T. viride* in fermentation medium containing various salts and olive oil. They found maximum growth rate between 12 and 48 hrs. of incubation. After 72 hrs there is slight decrease in enzyme production (overshoot and collapse). The activity of extracted crude lipase enzyme was observed 52U from *T. viride* and 49 U from *A. oryzae* (Table : 3,4). Lipase was purified from the culture broth by ammonium sulphate fractionation and acetone precipitation. In the process of ammonium sulphate fractionation, insoluble substances with lipase activity was not precipitated by centrifugation, but floated in the solution. This phenomenon was earlier reported for mono and diacylglycerol lipase and probably arose because of olive oil remaining in the culture broth (Toida *et al.,* 1995). Acetone precipitation was done next to remove any remaining olive oil and coloured material that might be affect the purification of enzyme.

The crude lipase enzyme from *Trichoderma viride* shows enzyme activity 49 IU/ml, while 35% purified enzyme shows activity 51 IU/ml. Further increase in the purity at 60%, the enzyme activity observed was 52 IU/ml. The dialyzed sample shows maximum enzyme activity *i.e.,* 55 IU/ml. The lipase enzyme extracted from *Aspergillus oryzae* shows higher enzyme activity than lipase extracted from *Trichoderma viride.* The crude enzyme, 35% 60% and dialyzed sample shows enzyme activity 54, 54, 56 and 58 IU/ml respectively (Table 4).

In *Aspergillus oryzae* and *Trichoderma viride,* the protein content in mg per ml as well as total lipase activity was decreased as increase in the purity of the enzyme (Table 3, 4). The noted results in the present investigation are similar to that of Kamimura *et al.,* (1999) who reported the maximum lipase activity achieved in 10 hours; however, Burket *et al.,* (2004) in *Geotrichum* sp. observed maximum lipase activity in 48 hours.

Table 1 : Optical Density at 410 nm of fungal cultures at the interval of 24 hours.

Interval Period	OD for *A. oryzae*	OD for *T. viride*
24	0.338	0.338
48	0.372	0.351
72	0.390	0.334
96	0.422	0.334
120	0.113	0.025

Table 2 : Absorbance of the enzyme at 410 nm.

Purification of Enzyme	*A. oryzae*	*T. viride*
Crude Enzyme	0.406	0.342
35% of Purification	0.410	0.358
60% of Purification	0.423	0.361
Dialysis	0.436	0.376

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Table 3 : Purification and activity of extracellular Lipase from *Aspergillus oryzae*.

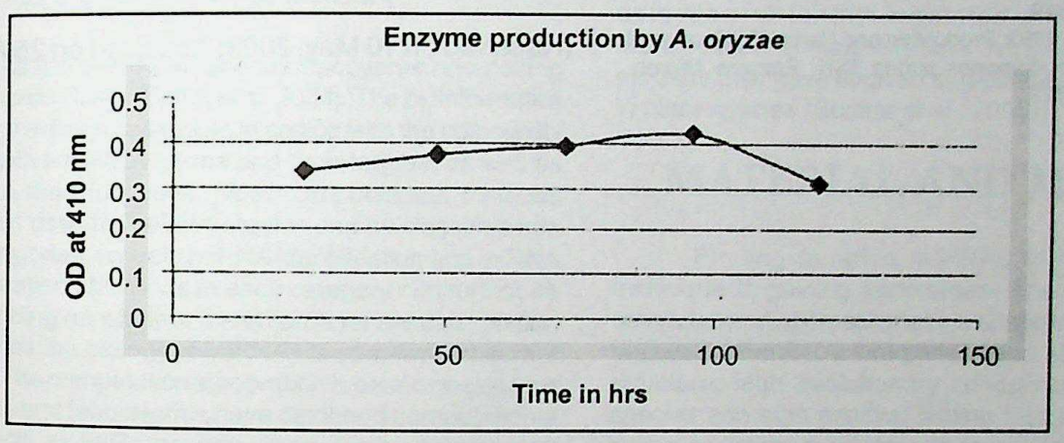
Enzyme	Volume (ml)	Lipase Activity (IU/ml)	Protein mg/ml	Total Lipase activity (IU)
Crude enzyme	100	54 ± 0.03	1.602 ± 0.02	5400 ± 0.4
35% Purification	80	54 ± 0.07	0.385 ± 0.06	4320 ± 0.9
60% Purification	79	56 ± 0.01	0.380 ± 0.03	4424 ± 0.7
Dialyzed sample	74	58 ± 0.06	0.372 ± 0.01	4292 ± 0.2

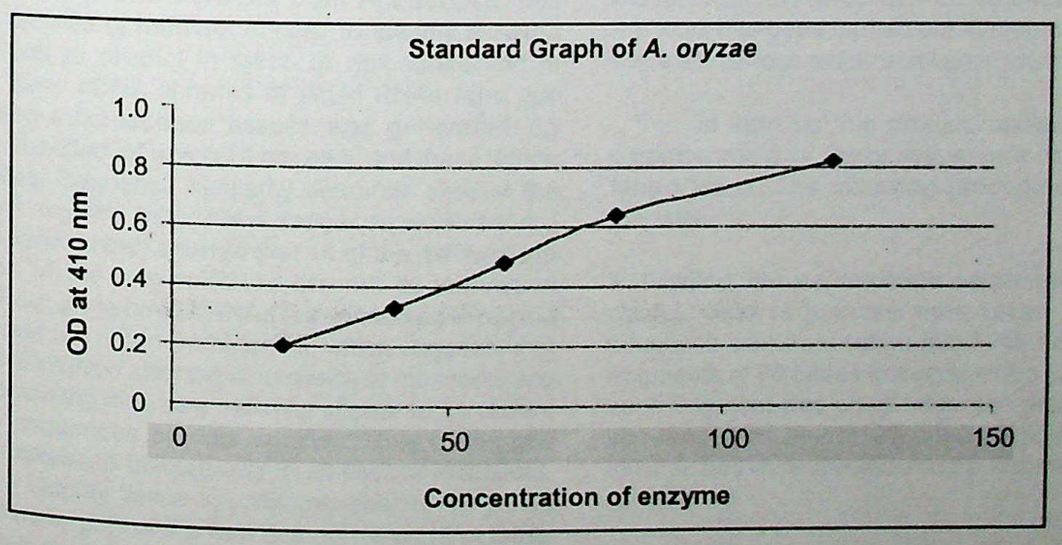
Values are mean ± SE.

Table 4 : Purification and activity of extracellular Lipase from *Trichoderma viride*.

Enzyme	Volume (ml)	Lipase Activity (IU/ml)	Protein mg/ml	Total Lipase activity (IU)
Crude enzyme	100	49 ± 0.01	1.453 ± 0.02	4900 ± 0.2
35% Purification	78	51 ± 0.06	0.363 ± 0.06	3978 ± 0.8
60% Purification	75	52 ± 0.03	0.352 ± 0.07	3900 ± 0.1
Dialyzed sample	72	55 ± 0.09	0.352 ± 0.01	3960 ± 0.4

Values are mean ± SE.





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Putative miRNA Detection in Rice Chromosome 8

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MicroRNAs (miRNAs) are a new class of non-coding 20-24 nt small RNAs. MicroRNAs (miRNAs) are present in both coding as well as non-coding regions of the whole genome. The computational strategy may not surely detect miRNAs but still the putative miRNAs detected using this approach is time-saving and economical. miRNAs are regulatory elements and regulation can not be studied *in silico* therefore, computational strategy can only give the potential candidates. Various tools and databases were *i.e.*, NCBI Map Viewer, Validation of miRNA and determination of regulatory roles can be done *in vitro* only.

Keywords : miRNA, NCBI, Chromosome 8.

INTRODUCTION

miRNAs are a class of recently discovered non-coding endogenous small RNAs (Bartel *et al*, 2004). The bioinformatics approaches have been invaluable in coping with the complexity of finding putative miRNA genes and their targets, as well as in deciphering their functions. Most computational methods that have been used for miRNA studies can be classified into two broad categories, namely miRNA identification and miRNA target identification . Methods in each category can further be divided depending on whether a method is for animals, plants, or viruses, since the biology of miRNAs is somewhat different in each case. The computational approach is based on genome sequence. Several laboratories have designed computational programs, such as MIRscan (http://genes.mit.edu/mirscan/) (Lim *et al.*, 2003). Computational analysis based on conservation with known miRNAs from Arabidopsis has predicted 20 families of miRNAs in rice. To identify miRNAs that are difficult to predict in silico or not conserved in Arabidopsis, three cDNA libraries of small RNAs from rice shoot, root and inflorescence tissues was generated. 35 miRNAs was identified, of which 14 are new, and these define 13 new families. Sequence similarity searches against the central miRNA registry (http://www.sanger.ac.uk/Software-/Rfam/mirna/search.shtml) showed that 14 of the miRNAs are new. Thirteen of the new miRNAs are not conserved in Arabidopsis. Four of the new miRNAs are conserved in related monocot species but not in Arabidopsis, which suggests that these may have evolved after the divergence of monocots and dicots. The remaining nine new miRNAs appear to be absent in the known sequences of plant species. Most of the rice miRNAs are expressed ubiquitously in all tissues examined, whereas a few display tissue-specific expression. 46 genes was predicted as targets of the new rice miRNAs: 16 of these predicted targets encode transcription factors, and other target genes appear to play roles in diverse physiological processes. Four target genes have been experimentally verified by detection of miRNA-mediated miRNA cleavage. The identification of new miRNAs in rice suggests that these miRNAs may have evolved independently in rice or been lost in other species (Sunkar *et al.*, 2005).

MATERIAL AND METHODS

Finding putative miRNAs through computational technique is gaining importance. There are 3 principles of computational approach that are based on major characteristic features of miRNA: hairpin-shaped stem loop secondary structure, high evolutionary conservation from species to species and high minimal folding free energy index (MFEI). However, plant miRNAs are less conserved than animal miRNAs. In the current project, *Oryza sativa* was taken as model organism which has 12 chromosomes. In this project, the work has been carried out in chromosome no. 8. Though there are various online softwares available to predict miRNA.

To start up this project, nucleic acid sequences of chromosome 8 of *Oryza sativa* were downloaded from NCBI Map Viewer. The following procedure was then followed stepwise:

1. Finding the palindromic sequences: The downloaded nucleic acid sequences were taken as input. This DNA sequence was transcribed into RNA and then were cut into fragments of 80 bases in length with a jump of 5 bases. Then each fragment was checked for the presence of palindromic sequences of length 20 base at both terminals. Only 4 mismatches were allowed and the fragments that satisfied this condition.

2. Secondary structure prediction: File containing the palindromic sequences was fed to RNAFOLD, which predicted

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List 1 : Putative miRNA in chromosome 8

GCGCGAGCGAGCGCUCCACCGGCUGGCCGGCUUGCGGCGGCGGUAGUCAGGAGCGCGGCGGCUGCGGUGCACGCUGGCGG
Energy : - 41.80
Beginning = 136316 End = 136396

UAGCGGACACUGCCCGGUCUUAAACUUGGGUUGUUUUCGUUGAGAGACAGCCUACAUUUAAGACCGGGCAGUGUCCACUA
Energy : - 55.70
Beginning = 312131 End = 312211

GUGGCGCUGUAGUUCGGCAGGUUGUAGGAGGAGCUGCCGCUCACCUGGCGGUUCCACAGCCUGAAGCCCGACAGCGACCG
Energy : - 42.20
Beginning = 492506 End = 492586

GCCCUCCUUCCCCUACUGCCCACCCGGGGUGGGCAGUCGCUCCCACCGCCCGCCCGGGCGGGCAGUCGCCGCGCGCGCGC
Energy : - 41.20
Beginning = 617881 End = 617961

AAGCGAGGCGGCGGCGGCGGCGGCGGCUUACUGUAGGACAGCGCGAGGCCGCAGCGGCCCGCGACGGCGCCGCGCGA
Energy : - 46.30
Beginning = 789416 End = 789496

GCGGCGGCUGCUGCUGCCGGAGCGGAUGAUGAGGCGGCGGCGGUGCUCCGACGAGCCGCCACGGUUGCGGCCGUGGCUGC
Energy : - 44.20
Beginning = 851586 End = 851666

CCGCCACGGUUGCGGCCGUGGCUGCUGCUGGACACCUCGAAGCGCGCCAGCCGACGCGGCGGUGGCGGCGGCGGCGG
Energy : - 44.70
Beginning = 851641 End = 851721

CCGGCGGUUUAGGCGGCGGCGCGCGCCGGCCGCUGCACGCCGCGUACGUGCAUGUGGCGCGCGGCGGCCGCCACCACACG
Energy : - 49.60
Beginning = 1017896 End = 1017976

CGCGGGCCGGCGUAGGCGGCGCCGGCGGAGGUGCGGACCGGUACGGGGGUGCGAGCCGGUGUAGGCUGCGCCGGCGGGCG
Energy : - 52.40
Beginning = 1068426 End = 1068506

CGCCGCUGCAGGCGCUAGACGACGCGGCGCUCGCGGCGCAGGCAGCUGCGGUCGCGGCUCUUCUUGCGCGGGCGGUAGCG
Energy : - 46.90
Beginning = 1083986 End = 1084066

CGCCGGAGGAGCCGAAGGGCUUCCGCUAGGCGCCGAAGAGCGGCCAGUCUAAGGGCGGAGGCCCGGAGACGCCGGCGGCU
Energy : - 46.70
Beginning = 1163201 End = 1163281

UCUACUCCCAGUAGCCACUGCCCGAGGUUUAUACCGGGCUAACUCUACUAAAAUUUCGGGCAGUGGCUACUGAGUAUAGC
Energy : - 42.80
Beginning = 1355161 End = 1355241

GCGGCGGCGCAGCGCGAGGGGGCUCCAGGUGAGGAGAGGACGGCCUCUUCGCCCGGAUCCGCUUCUCGUCGCGGUACCGC
Energy : - 45.60
Beginning = 1578861 End = 1578941

GGCGCAGCUGAGGCUCCGGCCGGUGUGGCUCGAGAUGCUGCUGGCGCUGCCGGUCGAGGGGACGCGGCCGCAGCGGCGGC
Energy : - 43.70
Beginning = 1591091 End = 1591171

UCGUAAAGGGUAUAAGUAUAACUACAAUUACUUAGAUCUAUAGAUCUAAGUAAUUGUAGUUAUACUUAUACCCUUUAUGA
-important
Energy : - 56.90
Beginning = 1703176 End = 1703256

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GCGAGCCAGAGCGUCGGAGCGGCGUGCUGUGGUGCGCCAGAGCGUCGGUAGCAGCACGUUGCCAACACGCUCUAGCUGUC
Energy : - 41.90
Beginning = 1722091 End = 1722171

Table 1 : Match miRNA in Rice Chromosome 8

Accessions	ID	Description	Chromosome	Sequences
MI0001101	osa-MIR 160f	*Oryza sativa* MIR160f stem-loop	8	GGAUUAACGCUGCCUGGCUCCCUGAAUGCCAUC CGAGAAGCGUGCCGCUGUGGCCGGCUGCUUCCU GGUUGGCAUUGAGGGAGUCAUGCAGGGUUUGCUC
MI0006967	osa-MIR 1427	*Oryza sativa* MIR1427 stem-loop	8	UGGCUGCGCGCGACCGCGCCGUCCCGCAGGCCAGC GCCGCGCCACCGCGCGGUUCCGCAGCAGGGACAUG CGCCACCCGUUACAGCCAACUGAAAUCUGCACUGA UUUGAUUACAUGCGCACGUAUAUAUGCAGUGCAUG CAGCGAGUGUGCGCUGUUCCUGGAUUCGGUCGCGC GUGGCCGUGCUGCGGAACCGUGCGGUGGCGCGGCG CCGGCCUGCGGGACCGCGCAGUCGCGCGCAGCCAAG CCACGGGACCG
MI0001437	osa-MIR 417	*Oryza sativa* MIR417 stem-loop	8	UUUGGUACAAAUUUGAAUUCUUAAAUCUCUUAUAU UAUGAUAUGAAUGUAGUGAAUUUGUUCCAUG
MI0000658	osa-MIR 156f	*Oryza sativa* MIR156f stem-loop	8	AGUUGACAGAAGAGAGUGAGCACACAGCGGCCAGAC UGCAUCGAUCUAUCAAUCUUCCCUUCGACAGGAUAGC UAGAUAGAAAGAAAGAGAGGCCGUCGGCGGCCAUGGA AGAGAGAGAGAGAGAGAGAUGAAAUGAUGAUGAUGAU ACAGCUGCCGCUGCGUGCUCACUUCUCUUUCUGUCAGCU

the Minimum Free Energy (MFE) for all the sequences files that contained secondary structure of each sequence.

3. Filtration of sequences based on energy : To filter out sequences based on the energies calculated by RNAfold. Only those sequences were considered as putative miRNA whose MFE was less than - 40 kcal.

4. Verification of known miRNAs with miRBase: The putative miRNAs were aligned against the known miRNA using BLAST available in miRBase database and putative miRNA sequences were verified against known miRNA sequences.

RESULTS AND DISCUSSION

MicroRNAs (miRNAs) are presented in both coding as well as non-coding regions of the whole genome. Taking this fact in consideration, whole genome sequences of chromosome 8 of *O. sativa* were downloaded from NCBI Map Viewer.

The sequences of chromosome 8 were first chomped to make its content in a single line. The program first transcribed the whole sequence and then cut them in the length of 80 bases with a jump of 5 bases. It then checked the first 20 bases with last 20 basis for matching. 4 mismatches were allowed. Only those sequences with matches greater than 15 bases were considered as palindromic sequences and were stored in a separate file. These sequences were then fed to RNAfold program. Secondary structure of the palindromic sequences was calculated using RNAfold available in ViennaRNA package. It gave energy of each folded structure and the results was stored. Filtered out the final putative miRNAs based on energy. Only those miRNAs were considered as putative miRNAs whose energy was less than - 40 kcal. (List-1).

The above procedure resulted in detection of putative miRNAs of chromosome 8 and then the results were aligned with the known miRNAs of the corresponding chromosome in miRBase. However, not all miRNAs in miRBase from *Oryza sativa* could be verified using this method.

BLAST was performed and then the results was analysed. Four hits (osa-MIR160f, osa-MIR1427, osa-MIR417, osa-MIR156f) (Table-1) from the results matched with the known miRNA in chromosome 8. The computational strategy may not surely detect miRNAs but still the putative miRNAs detected using this approach is time-saving and economical.

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Extraction and Characterization of Tocopherol from Sunflower and Soybean Oil

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Tocopherol or vitamin E is a fat soluble vitamin which exists in eight forms, as an antioxidant. Natural vitamin E exists in eight biological active forms or isomers, four tocopherols and four tocotrienols. The most abundant sources of vitamin E are vegetable oils extracted from sunflower, corn, soybean and olive oil. Most natural vitamin E supplements are now derived from vegetable oils usually soybean oil. The extracted tocopherol was quantified and characterized with IR Spectroscopy, NMR spectroscopy and UV- visible spectroscopy. The tocopherol (*i.e.,* vitamin E) was isolated from sunflower and Soybean oil. The IR spectrophotometric detection shows that, cyclic ether (1236-11cm^{-1}), phenolic -OH (2926 cm^{-1}), C-H stretching of *cis* CH_3 (723.3 cm^{-1}). A sharp weapon NMR spectrophotometer confirmed peaks due to primary alkyl group proton and phenolic proton.

Keywords : Vitamin E, Tocopherol, IR, NMR, BHT (butyl hydroxylated tolune).

INTRODUCTION

Vitamin E (tocopherol) was first isolated in 1936 from wheat germ oil. Historically, vitamin E was known for it's essential role in maintaining pregnancy, as the name indicates. "Tocopherol" derives from the Greek words *tocos* (to birth) and *pherein* (carry) (Eitenmiller and Landen, 1994). Vitamin E refers collectively to natural and synthetic derivatives of the tocopherols and tocotrienols. Each form shows different bioactivities (Pryor, 1995). Other results from *in vitro* and *in vivo* measures of antioxidant activity have identified alpha tocopherol as the most active antioxidant, which correlates with measures of the bioactivity (Burton and Ingold, 1989). The vitamin E compounds are freely soluble in lipids and organic solvents such as hexane, but are practically insoluble in aqueous solutions. Vitamin E is most recognized today as the principle lipid-soluble antioxidant in both plant and animal systems (Diplock, 1983; Fryer, 1992), which shows crucial participation in defenses against oxidative stress. Antioxidants such as vitamin E act to protect cells against the effects of free radicals, which are potentially damaging by products of energy metabolism. Free radicals can damage cells and may contribute to his development of cardiovascular disease and cancer (Packer and Landvik, 1989). Vitamin E has also been shown to play a role in immune function, in DNA repair and other metabolic processes and shows enhancement in immune surveillance and suppressing tumor growth (Factor *et al.,* 2000).

Tocopherols are sensitive to oxygen, light, and temperature and can be oxidized by heat, light, oxygen, or other oxidizing conditions. Therefore care must be taken to avoid their degradation (Eitenmiller & Landen, 1994). In present investigation specific protocols have been developed to minimize loss of vitamin E during extraction and analysis. Alternative methods are commonly employed because the heat and alkali conditions called for in saponification can result in substantial degradation of tocopherols (Ueda and Igarashi, 1987).

In foods, the most abundant sources of vitamin E are vegetable oils such as palm oil, sunflower, corn, soybean and olive oil. Nuts, sunflower seeds, sea buckthorn berries, kiwi fruit and wheat germ are also good sources. Other sources of vitamin E are whole grain, fish, peanut, butter and green leafy vegetables. Although originally extracted from wheat germ oil, most natural vitamin E supplements are now derived from vegetable oils usually soybean oil.

Several methods have been developed to extract vitamin E components namely supercritical fluid extraction, solvent extraction, and Soxhlet extraction. Microwave extraction is a newly developed extraction method (Eskilsson and Bjorklund, 2000; Ondruschka and Asghari, 2006; Zigoneanu (2006). In the present study new protocol was developed for the extraction of tocopherol (*i.e.,* vitamin E) from soybean and sunflower oil. The biochemical and analytical methods (spectrophotometry, IR and NMR) were used for detection of extracted tocopherol.

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Table 1 : Infra-red spectroscopy (IR) Analysis of Sunflower oil:

Sr. No.	Frequency (in cm^{-1})	Type of functional group
1.	1745.64	Aromatic C double bond C
2.	2926.11	OH-phenolic
3.	1236.41	Ar-O-R (Ether)
4.	2854.74	C-H Stretching
5.	1653.05	Aromatic proton
6.	3007.12	Free-OH
7.	723.33	*Cis* R nature of C-H stretching of CH_3

Table 2 : Infra-red spectroscopy (IR) Analysis of Soybean oil:

Sr. No.	Frequency (in cm^{-1})	Type of functional group
1.	1745.64	Aromatic C double bond C
2.	2926.11	OH-phenolic
3.	1236.41	Ar-O-R (Ether)
4.	2854.74	C-H Stretching
5.	1653.05	Aromatic proton
6.	3007.12	Free-OH
7.	723.33	*Cis* R nature of C-H stretching of CH_3

Table 3 : Nnuclear magnetic resonance (NMR) Analysis of Soybean oil:

Sr. No.	Peaks assigned	Types of protons
1.	A, B, D	Primary alkyl R-CH_3
2.	G	Primary alkyl R-CH_3
3.	H	Secondary alkyl R-CH_2
4.	F	Secondary alkyl R-CH_2
5.	E	Secondary alkyl R-CH_2
6.	-	May be due to petroleum ether
7.	L	
8.	C	Phenolic , Ar-OH

Table 4 : Nnuclear magnetic resonance (NMR) Analysis of Sunflower oil:

Sr. No.	Peaks assigned	Types of protons
1.	A, B, D	Primary alkyl R-CH_3
2.	G	Primary alkyl R-CH_3
3.	H	Secondary alkyl R-CH_2
4.	F	Secondary alkyl R-CH_2
5.	E	Secondary alkyl R-CH_2
6.	M	
7.	J	
8.	C	Phenolic , Ar-OH

UV-visible spectrophotometer showed λ max of extracted tocopherol 310 and 320 nm from soybean and sunflower oil respectively. The observed values are matching with therotical figures (Williams and Fleming, 2006).

In IR spectrophotometric detection shows that, cyclic ether (1236-11cm^{-1}), phenolic -OH (2926 cm^{-1}), C-H stretching of *cis* CH_3 (723.3 cm^{-1}). A sharp weapon NMR spectrophotometer confirmed peaks due to primary alkyl group proton and phenolic proton. Extracted vit. E. can be used in industry as an inexpensive preservative. It has wide use in encouraging skin healing and reducing scarring after injuries.

A high performance liquid chromatography (HPLC) method was developed for the quantitative determination of tocopherol in different seed oils by Fazli Manan *et al.,* (1994). After saponification the oil samples and extracts in n-hexane, tocopherol vitamers were quantitatively analyzed by HPLC with fluorimetric detector. Assay of tocopherol vitamers added to margarine resulted in approximately 97% recovery, showing high precision of the method. The tocopherol vitamers were eluted isocratically by an eluent containing 95% hexane, 5% diethyl ether at a pump flow rate of 1.5ml/min.a silica column (*Si*-60) with temperature of 45°C was used.

In normal phase chromatography, the forms of vitamin E are separated based on the degree of methylation of the chroman ring and are eluted in order of increasing polarity (Tan and Brzuskiewicz, 1989). The obtained from IR and NMR analysis was similar to the result recorded by Kramer *et al.* (1999). They used a normal phase diol column to separate tocopherols with good results. Through NMR analysis they determined that the stationary phase was not a diol but actually an epoxide. The epoxide structure gives the column stability to polar and nonpolar solvents and moderate polarity. They report that the diol column was stable over hundreds of assays and results were reproducible (Kramer *et al.,* 1999).

MATERIAL AND METHODS

Material: Commercially available Soybean and sunflower oil was used for the isolation and extraction of tocopherol. All other chemicals and regents used were of Analar grade.

Saponification & extraction of tocopherol : A weighed quantity of oil samples (50 ml) was taken in 250 ml flask covered with aluminum foil. Nitrogen gas was flushed and a freshly prepared 50 ml 8% Ethanolic KOH solution; 5 ml petroleum ether and 10 ml absolute alcohol were added to the flask. 20 mg BHT (butyl hydroxylated toluene) was added as an antioxidant to prevent the oxidation of tocopherol. Nitrogen gas was again flushed in the flask and stoppered and swirled gently to mix the reagents.

The contents of the flask were saponified under reflux on boiling water bath for 30 mins. The flask was then cooled to room temperature. Diethyl ether (100 ml) was added to the

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flask with constant shaking. The contents were then transferred to a 500 ml separating funnel. Following separation of the 2 layers, the lower aqueous layer was discarded. The ether layer was washed with 100ml distilled water. After evaporating the mixture it was dissolved in 100 ml n-hexane.

The sample was then employed for the analysis by IR, NMR, and UV-visible spectroscopy. The IR specta were recorded on chemmitto FTIR-435 instrument of Shimadzu Make. The NMR were recorded on varian mercury YH-300 MHz using $CDCl_3$ in the chemistry research laboratory of IIT, Mumbai.

RESULTS AND DISCUSSION

From sunflower oil practical yield of tocopherol was found to be 16% and from soybean oil was 24% starting from 50 ml sunflower oil and soybean oil practical yield was 8 ml and 12 ml respectively. No decolourisation of $KMnO_4$ indicates saturated character of vitamin E. Presence of phenolic ring was confirmed by preparation of sodium salt of phenol. Alternatively it can be confirmed using NaOH + $FeCl_3$ mixture gives phenolic red colour.

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Role of Environment in Selection of Mulberry Genotypes for Crop Improvement

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The choice of selection environment is one of the most debated aspects in plant breeding. In general, testing in favourable (Non-stress) environments favours expression of genes and selection in poor (stress) environments indicates adaptability. The success of selection in developing a high yielding variety in mulberry, like in other agriculture crops, depends on proper strategy of screening and selection. In the present study, a large hybrid population of 1152 hybrids generated from 35 crosses was screened initially under non-stress and thereafter under stress conditions. Depending upon mean yield and standard deviation in the respective environments selection was made. These two sets of data were compared and the genotypes with high mean yield and large yield deference were selected for the next stage. Of the genotypes screened in the first stage of selection in non-stress and stress environments, 42 High yielding genotypes were selected. The selected genotypes (42) were raised in bigger plots along with local checks and evaluated for two consecutive years involving five crops per year. Finally, 13 genotypes yielding above average were selected. Stability analysis indicated differential response among selected genotypes to different seasons suggesting the scope for selection of productive genotypes for different environments. The data suggested that preliminary selection under non-stress conditions alone would be sufficient in selection of promising adaptable productive mulberry genotypes.

Keywords : Mulberry, Selection environment, Stability analysis.

INTRODUCTION

The initial testing environment seldom matches with the agricultural practices. The environment in which plants are grown will affect the expression of certain morphological and physiological characters and thus affect the ease with which they can be selected. Selection is generally aimed at identifying genotypes that have high average value for a number of environments. But it is quite impossible to conduct multi-locational trials of a large number of genotypes at different sites.

Hence, the choice of selection environment is one of the most debatable aspects in plant breeding. In mulberry, there is no such documented evidence, which indicates the relative response of the plants to selection in stress and non-stress environments. While Gotoh and Osanai (1959) found that wheat selection is efficient in poor environment with low fertility, Byth *et al.*, (1969) did not find any difference between non-stress and stress environments while working with Soybean. Similar work was done on Oats by Frey (1964) who observed that selection was equally efficient in both stress and non-stress environments. In maize, Arboleda-Riviera and Compton (1974) indicated that direct selection in non-stress environment was not associated with a significant advance in stress environment. In rice, Oka and Chang (1964) favoured selection under non-stress conditions for increased response to weeding and adequate fertilizer, and it gave rise to genotypes productive under stress conditions.

MATERIAL AND METHODS

A large variable population of 1152 hybrids derived from 35 crosses involving 24 diverse parents of both indigenous and exotic origin were considered for the study. The population thus raised was allowed to establish in the field with care to avoid loss of any genotype. After a year of establishment, the plants were pruned during the month of May and maintained as per the recommended practices for cultivating mulberry under irrigated conditions, including providing 1.5-acre inch irrigation every 8-10 days during non-rainy days. The period of growth between May and July, most favourable for growth of mulberry, was considered as non-stress environment. The leaf yield was recorded by plucking the leaves of individual plants after 70 days of growth.

After the harvest in non-stress environment, the plants were pruned and the stress conditions were created by withdrawing all inputs like fertilizers, irrigation etc. The plants were maintained under such conditions and pruned at the end of December. The pruned plants were allowed to grow for a period of 70 days under the stress conditions. Neither irrigation was provided to the plants nor there were any rains during the growth period (December-February). This was considered as

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stress environment. The leaf yield was recorded by plucking leaves from each individual plant. The yield data of each individual plant in non-stress and stress environments were compared. Genotypes recording high yield in both the environments were short listed through upward selection based on yield+SD in the respective environments.

The genotypes thus selected were multiplied and planted in augmented randomized design along with the check variety (S36) for yield testing. The experimental plot was maintained under optimum irrigated conditions and provided with recommended doses of nutritional inputs. The genotypes were evaluated for two years after establishment. Data of five leaf harvests were recorded during each year and analyzed as per the method suggested for augmented design by Federer (1956) to select genotypes significantly superior in yield over the check. The five seasonal data averaged over two years were subjected to joint regression analysis and b value was calculated as per Finlay and Wilkinson (1963).

RESULTS AND DISCUSSION

Initially the performance of each genotype was evaluated in both stress and non-stress environments (Table 1). While the average leaf yield per plant under non-stress conditions was 351.80 g, it reduced to 106.17 g under stress. The Mean+SD value of the population in non-stress environment was as high as 252.06 g in comparison to 88.55 g under stress. The genotypes recording leaf yield of over 603.86 g/plant (351+252.06) in non-stress environment and those recording over 194.72 g/plant (106.17+88.55) under stress were selected. The two sets of data were compared to short list 42 genotypes. While most of the 42 selected genotypes figured in list of genotypes selected in the two environments there relative ranking among the genotypes differed between the two lists indicating that performance of a genotype in non-stress environment need not necessarily indicate its relative performance in stress environment. Arboleda-Riviera and Compton (1974) in their study on grain maize reported that selected in poor (stress) environment is

Table 1 : Computation of environmental index from 42 genotypes and two check genotypes grown in five seasons (Av. of two years).

Season	Months of leaf harvest	Mean leaf yield (g/Plant)	Environmental index
1.	March-April	454.67	-81.989
2.	May-June	558.15	+21.489
3.	July-August	615.39	+78.727
4.	September-October	581.39	+45.045
5.	December-January	473.39	-63.273

Table 2 : Annual seasonal mean yield and regression co-efficient (b-value) of selected genotypes during second stage of selection.

Sl.No.	Genotype number	Annual mean leaf yield (g/plant)	'b' value	Rank in non-stress environment	Rank in stress environment
1.	S-36(check)	2727	0.862	-	-
2.	K2(check)	2492	0.816	-	-
3.	1138	5748	2.074	1	2
4.	416	5150	1.693	2	11
5.	519	4923	1.682	3	1
6.	113	4790	1.485	4	4
7.	995	4688	2.221	5	40
8.	63	4666	1.972	-	-
9.	566	4629	1.398	7	8
10.	903	4547	1.524	8	31
11.	432	4471	1.497	9	9
12.	1082	4260	1.733	10	28
13.	144	4231	1.456	11	6
14.	76	4078	1.908	12	15
15.	87	3945	2.772	13	3

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more efficient for the situation and induced a closer correlated response in good (non-stress) environment. Direct selection in non-stress environment was not associated with significant improvement and there is no reason to expect a general answer. With specific reference to selection in stress and non-stress environments, Ceccarelli (1994) showed that the average yield of the selected Barley lines in non-stress environment was almost four times than that of the stress environment. Similar trend was observed in the present study where the average yield in non-stress environment was almost three times the yield under stress.

The present study was aimed at understanding whether initial selection of mulberry genotypes suitable for stress environments would be possible through selection in non-stress environment. The main reasons for selection in non-stress environment are the difficulties faced in maintaining the genotypes in stress environment and the chances of loosing potential genotypes to adverse environmental conditions during the establishment stage. The remarks of Ceccarelli (1994) that introduction of inputs like fertilizers and irrigation is an essential pre-requisite for successful breeding programmes is also in support of the present view.

In the second stage of selection, the 42 selected genotypes were maintained in non-stress environment and evaluated for two years, against control genotype. The yield data of ten harvests over a period of two years indicated that 13 genotypes recorded significantly higher leaf yield over the check. The increase in leaf yield over the check was in the range of 44.77-110.94%. All the 42 genotypes and the checks were subjected to joint regression considering the seasons as different environments. The stability parameter (b-value) were calculated from the data and it was observed that all the 13 genotypes recording leaf yield higher than the checks showed b-values above 1.00 (Table-2), indicating their response to the high yieling conditions (non-stress). When the performance of the 13 selected genotypes was compared to their performance under stress during the first stage, it was noticed that 10 out of the 13 performed extremely well under stress conditions.

It could be inferred from the present study that in mulberry, preliminary selection under stress and non-stress conditions yield more or less similar results. This being the case, it is suggested that the preliminary selection be made under optimum conditions, as expression of genetic potential is maximum under optimum conditions. This would also avoid loss of precious genetic material to adverse environmental conditions during the establishment stage, save time, energy and resources. The short listed genotypes could then be considered for evaluation under different environments to identify varieties suitable for specific situations.

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Studies on Induced Micro-Mutations in Cowpea (*Vigna unguiculata* (L.) Walp.)

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Mutagenic effects of gamma rays, ethidium bromide and ethyl methane sulphonate separately as well as in combinations were studied in M_1 generation and in M_2 generation of cowpea genotype CO VU 623. The magnitude of induced genetic effect was comparable with physical, chemical and in combination of physical plus chemical mutagens, causing a similar effect in the expression of polygenic characters. There were no relation between the dose of the mutagen and the mean expression of the traits studied as well as the extent of variability induced. Total number of pods per plant was the highly responding character. Of all the traits studied, number of pods per cluster was the least responding character. The higher values of predicted heritability and genetic advance suggested that the mutations might have mostly occurred at loci having additive effects.

Keywords : Gamma rays, Ethidium bromide, Ethyl methane sulphonate and Cowpea.

INTRODUCTION

Cowpea crop being autogamous in nature is likely to lose its variability due to continuous selection pressure. Mutation breeding creates a new unexplored and unexploited variability. Effects of the mutagenic factors on polygenes and its subsequent manifestation is aptly called as micromutations (Gaul, 1958). The present study was formulated to induce micro-mutation in cowpea genotype CO VU 623 with gamma rays, EB, EMS and combination of gamma rays with EB and EMS.

MATERIAL AND METHODS

Cowpea (*Vigna unguiculata* (L.) Walp.) genotype CO VU 623 was used for the present study. Dry seeds of uniform size were exposed to 400, 450, 500, 550, 600, 650 and 700 GY gamma rays, using a 1000 ci ^{60}Co gamma chamber at a dose rate of 54.05 rads/sec. Seeds were also treated with 0.08, 0.10, 0.13, 0.15, 0.18 and 0.20 mM EB and 20, 30 mM EMS solutions prepared in double distilled water, adjusted to pH 7.0 for 6 h, with intermittent shaking at 21 ± 1°C. The combination treatments were performed by treating the dry seeds with gamma rays first and soaking the seeds in the mutagen solution for 6 h. Exclusive controls (soaked in double distilled water for 6 h and 12 h) were also included. The seeds treated with mutagens, along with untreated control seeds (dry, soaked for 6 h and 12 h) were sown immediately in the field.

Sixty seeds from each of the treatment along with the control were sown in a Randomized Block Design replicated thrice. From the surviving M_1 plants, 10 plants which had no visible morphological changes were selected at random and harvested separately. All the seeds from the selected M_1 plants along with control were sown in the Randomized Block Design, replicated thrice. Each M_1 plant assumed a single family in the M_2 generation. A spacing of 45 x 15 cm was adopted. A two row plot size of three metre was allotted for each entry. Recommended agronomic practices and need based plant protection measures were followed. Observations were recorded on number of branches per plant, number of clusters per plant, number of pods per cluster, total number of pods per plant, number of seeds per pod, 100 seed weight and seed yield per plant (g), from 10 randomly selected plants that had no visible morphological changes in each family of every treatment in M_2 generation. Standard statistical methods were adopted.

RESULTS AND DISCUSSION

The induction of micro-mutations in M_2 generation was measured in terms of mean and variance. Broad sense heritability and the expected genetic gain through selection in the mutated population were also computed. The mean expressions of all the yield attributing traits were shifted towards negative direction (Tables 1-7). The means of the different treatments were observed on either sides of the control representing a bi-directional distribution of the mutated loci. Perhaps each treatment might have acted on a different gene or block of genes which are involved in the genetic pathway determining the morphogenetic potentiality of the exophenotypes.

Yield is the end product. It does not possess gene *per se* as such the *per se* performance was less compared to the control (Table 2). It may be due to the negative complementation among the unfavourably mutated alleles of the component characters. A similar trend was also observed by Suresh (1975)

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Table 1 : Components of variance for number of branches per plant in M_2.

Treatment dose/conc.	Mean ± SE	σ^2 g	h^2 (%)	GA as % of mean
Gamma rays (GY)				
400	3.50 ± 0.46	0.03	04.48	02.29
450	3.76 ± 0.25	0.13	39.39	12.50
500	3.23 ± 0.27	0.09	28.13	10.22
550	3.42 ± 0.37	0.22	33.85	16.37
600	3.53 ± 0.33	-	-	-
650	3.80 ± 0.23	0.16	50.00	15.26
700	3.89 ± 0.37	0.17	28.81	11.83
EB mM				
0.08	3.20 ± 0.27	0.08	25.81	09.38
0.10	3.80 ± 0.16	0.31	79.49	26.84
0.13	4.40 ± 0.25	0.18	47.37	13.64
0.15	3.59 ± 0.25	0.26	57.78	22.28
0.18	3.35 ± 0.34	0.30	46.15	22.99
0.20	3.63 ± 0.30	0.03	10.00	03.03
EMS mM				
20.00	3.44 ± 0.15	0.24	77.42	25.87
30.00	3.30 ± 0.24	0.10	35.71	11.82
Gamma rays + EB				
450 GY + 0.08 mM	3.75 ± 0.36	0.05	11.36	04.27
550 GY + 0.08 mM	3.32 ± 0.31	0.07	19.44	07.23
650 GY + 0.08 mM	3.85 ± 0.34	0.24	40.68	16.62
Gamma rays + EMS				
450 GY + 20 mM	3.60 ± 0.29	0.13	33.33	11.94
550 GY + 20 mM	3.89 ± 0.28	0.13	34.21	11.05
650 GY + 20 mM	3.70 ± 0.23	0.36	67.92	27.57
Control (dry seeds)	4.49			

'F' value	3.30**
SE	0.22
CD	0.63

Table 2 : Components of variance for number of clusters per plant in M_2.

Treatment dose/conc.	Mean ± SE	σ^2 g	h^2 (%)	GA as % of mean
Gamma rays (GY)				
400	8.61 ± 0.68	0.91	39.22	14.29
450	7.59 ± 0.89	0.42	15.00	6.85
500	9.28 ± 0.40	0.40	44.44	9.38
550	8.89 ± 0.34	0.29	43.28	8.21
600	9.77 ± 0.72	0.32	23.19	5.73
650	10.15 ± 0.41	0.85	62.50	14.78
700	9.42 ± 0.96	0.68	19.60	7.96
EB mM				
0.08	8.18 ± 0.72	-	-	-
0.10	10.45 ± 0.51	1.67	67.34	20.86
0.13	9.97 ± 0.62	0.57	32.39	8.93
0.15	7.06 ± 0.37	0.27	42.86	9.92
0.18	7.84 ± 0.35	1.38	78.41	27.30
0.20	7.14 ± 0.19	0.50	81.97	18.49
EMS mM				
20.00	8.43 ± 0.64	0.23	15.44	4.63
30.00	9.07 ± 0.44	1.16	66.29	19.96
Gamma rays + EB				
450 GY + 0.08 mM	8.88 ± 0.55	0.23	50.00	7.88
550 GY + 0.08 mM	8.30 ± 0.64	0.85	40.28	14.58
650 GY + 0.08 mM	7.41 ± 0.23	0.23	57.50	10.12
Gamma rays + EMS				
450 GY + 20 mM	8.14 ± 0.78	0.04	02.13	0.74
550 GY + 20 mM	9.22 ± 0.41	0.95	64.63	17.46
650 GY + 20 mM	9.51 ± 0.64	1.22	49.80	16.93
Control (dry seeds)	10.69			

'F' value	5.19**
SE	0.49
CD	1.41

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Table 3 : Components of variance for number of pods per cluster in M_2.

Treatment dose/conc.	Mean ± SE	σ^2 g	h^2 (%)	GA as % of mean
Gamma rays (GY)				
400	1.99 ± 0.06	0.0060	35.29	04.52
450	1.67 ± 0.04	0.0010	14.29	01.20
500	1.79 ± 0.04	0.0090	64.29	08.94
550	1.58 ± 0.06	0.0170	73.91	14.56
600	1.59 ± 0.06	0.0080	40.00	07.55
650	1.70 ± 0.04	0.0120	66.67	10.59
700	1.72 ± 0.08	0.0030	15.00	02.33
EB mM				
0.08	1.46 ± 0.10	0.0070	20.00	05.48
0.10	1.84 ± 0.08	0.0020	09.09	01.63
0.13	1.61 ± 0.05	0.0070	50.00	07.45
0.15	1.83 ± 0.06	0.0140	58.33	10.38
0.18	1.83 ± 0.06	0.0100	47.62	07.78
0.20	1.60 ± 0.06	0.0020	15.38	02.50
EMS mM				
20.00	1.72 ± 0.09	0.0280	50.91	14.53
30.00	1.36 ± 0.55	0.0003	04.29	00.74
Gamma rays + EB				
450 GY + 0.08 mM	1.52 ± 0.06	0.0001	01.11	00.13
550 GY + 0.08 mM	1.50 ± 0.07	0.0120	43.48	09.33
650 GY + 0.08 mM	1.53 ± 0.04	0.0120	70.59	12.42
Gamma rays + EMS				
450 GY + 20 mM	1.62 ± 0.06	-	-	-
550 GY + 20 mM	1.66 ± 0.08	0.0110	03.55	02.41
650 GY + 20 mM	1.47 ± 0.09	-	-	-
Control (dry seeds)	1.63			
'F' value	3.76**			
SE	0.09			
CD	0.22			

Table 4 : Components of variance for total number of pods per plant in M_2.

Treatment dose/conc.	Mean ± SE	σ^2 g	h^2 (%)	GA as % of mean
Gamma rays (GY)				
400	15.46 ± 1.21	30.26	87.25	68.43
450	9.64 ± 0.87	1.89	45.11	19.71
500	15.43 ± 1.63	2.09	20.71	8.55
550	15.54 ± 2.28	7.31	31.87	20.21
600	16.05 ± 2.00	1.85	13.31	6.36
650	14.84 ± 2.84	-	-	-
700	17.82 ± 2.01	14.46	54.22	32.38
EB mM				
0.08	12.06 ± 1.25	10.46	68.91	48.85
0.10	16.59 ± 1.52	15.60	68.97	40.75
0.13	14.11 ± 2.33	3.88	19.24	12.62
0.15	11.45 ± 1.37	2.81	33.25	17.38
0.18	9.18 ± 1.18	6.55	60.87	44.77
0.20	11.56 ± 1.62	-	-	-
EMS mM				
20.00	11.93 ± 0.92	4.37	63.15	28.67
30.00	13.48 ± 1.23	7.72	62.71	33.31
Gamma rays + EB				
450 GY + 0.08 mM	12.11 ± 1.13	14.32	78.81	57.14
550 GY + 0.08 mM	13.66 ± 1.79	29.18	75.13	70.64
650 GY + 0.08 mM	13.76 ± 1.40	2.27	27.58	11.85
Gamma rays + EMS				
450 GY + 20 mM	16.15 ± 2.17	7.59	34.86	20.74
550 GY + 20 mM	17.01 ± 1.40	36.00	85.92	67.37
650 GY + 20 mM	16.84 ± 2.46	10.25	35.90	23.46
Control (dry seeds)				
'F' value	28.28			
SE	12.22**			
CD	1.53			
	4.35			

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Table 5 : Components of variance for number of seeds per pod in M_2.

Treatment dose/conc.	Mean ± SE	σ^2 g	h^2 (%)	GA as % of mean
Gamma rays (GY)				
400	10.78 ± 0.41	1.72	77.48	22.08
450	09.32 ± 0.44	0.22	27.16	05.36
500	09.89 ± 0.40	0.60	32.97	09.30
550	10.37 ± 0.59	0.10	08.70	01.83
600	11.32 ± 0.41	2.08	80.31	23.50
650	10.03 ± 0.22	0.38	71.70	10.77
700	09.67 ± 0.49	1.55	67.98	21.82
EB mM				
0.08	10.32 ± 0.07	0.67	76.14	14.24
0.10	10.18 ± 0.40	0.45	47.37	09.33
0.13	10.58 ± 0.28	0.45	65.22	10.59
0.15	11.03 ± 0.54	0.95	51.35	13.06
0.18	10.11 ± 0.42	0.53	50.00	10.48
0.20	10.42 ± 0.36	1.45	77.96	21.02
EMS mM				
20.00	10.40 ± 0.40	0.21	26.92	04.71
30.00	10.60 ± 0.40	0.69	57.98	12.26
Gamma rays + EB				
450 GY + 0.08 mM	10.31 ± 0.30	0.89	75.42	16.39
550 GY + 0.08 mM	10.85 ± 0.40	0.46	45.54	08.66
650 GY + 0.08 mM	11.01 ± 0.30	1.05	77.21	16.80
Gamma rays + EMS				
450 GY + 20 mM	11.19 ± 0.36	0.77	65.81	13.14
550 GY + 20 mM	10.52 ± 0.78	-	-	-
650 GY + 20 mM	10.43 ± 0.70	0.51	50.50	10.07
Control (dry seeds)	12.79			
'F' value	6.03**			
SE	0.36			
CD	1.02			

Table 6 : Components of variance for 100 seed weight in M_2.

Treatment dose/conc.	Mean ± SE	σ^2 g	h^2 (%)	GA as % of mean
Gamma rays (GY)				
400	4.63 ± 0.20	0.13	52.00	11.66
450	4.60 ± 0.15	0.17	70.83	15.43
500	4.44 ± 0.30	0.003	00.97	02.25
550	4.33 ± 0.40	0.53	52.48	25.17
600	4.50 ± 0.29	2.16	38.10	11.33
650	4.49 ± 0.40	0.35	41.67	17.59
700	4.53 ± 0.30	0.20	40.82	13.02
EB mM				
0.08	4.26 ± 0.26	0.03	12.50	03.05
0.10	4.17 ± 0.36	0.01	01.67	05.76
0.13	4.42 ± 0.24	0.12	40.00	10.18
0.15	4.39 ± 0.70	-	-	-
0.18	4.44 ± 0.35	0.39	51.32	20.72
0.20	4.03 ± 0.17	0.12	57.14	13.40
EMS mM				
20.00	4.24 ± 0.12	0.06	54.55	08.73
30.00	3.98 ± 0.12	0.04	44.44	06.78
Gamma rays + EB				
450 GY + 0.08 mM	4.37 ± 0.20	0.08	40.00	08.47
550 GY + 0.08 mM	4.25 ± 0.37	0.15	26.32	09.65
650 GY + 0.08 mM	4.14 ± 0.19	0.24	68.57	20.29
Gamma rays + EMS				
450 GY + 20 mM	4.54 ± 0.16	0.09	52.94	09.9
550 GY + 20 mM	4.39 ± 0.25	-	-	-
650 GY + 20 mM	4.25 ± 0.30	0.14	31.82	10.24
Control (dry seeds)	4.86			
'F' value	2.00**			
SE	0.17			
CD	0.50			

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Table 7 : Components of variance for seed yield per plant in M_2.

Treatment dose/conc.	Mean ± SE	σ^2 g	h^2 (%)	GA as % of mean
Gamma rays (GY)				
400	5.80 ± 0.49	0.21	22.11	7.59
450	3.19 ± 0.30	0.15	30.00	12.85
500	5.12 ± 0.53	0.25	22.32	9.57
550	5.61 ± 0.98	1.25	29.69	22.28
600	5.69 ± 0.40	1.03	68.21	30.40
650	5.24 ± 0.55	1.08	54.00	29.96
700	5.96 ± 0.50	3.50	81.59	58.39
EB mM				
0.08	4.24 ± 0.45	1.93	75.10	58.49
0.10	5.39 ± 0.85	3.47	61.09	54.64
0.13	5.01 ± 0.90	0.92	25.92	20.16
0.15	4.24 ± 0.85	-	-	-
0.18	3.47 ± 0.47	0.62	47.69	32.28
0.20	4.02 ± 0.70	0.54	26.60	19.40
EMS mM				
20.00	4.23 ± 0.40	0.27	34.62	14.89
30.00	4.88 ± 0.54	2.04	69.62	50.20
Gamma rays + EB				
450 GY + 0.08 mM	3.89 ± 0.38	1.12	71.34	47.30
550 GY + 0.08 mM	4.70 ± 1.02	-	-	-
650 GY + 0.08 mM	4.74 ± 0.54	0.30	25.42	12.03
Gamma rays + EMS				
450 GY + 20 mM	6.02 ± 0.67	1.12	71.34	47.30
550 GY + 20 mM	6.09 ± 0.89	2.56	51.72	38.92
650 GY + 20 mM	5.46 ± 0.90	0.54	17.88	11.72
Control (dry seeds)	13.85			
'F' value	33.68**			
SE	0.53			
CD	1.51			

in greengram; Nadarajan *et al.* (1983) in redgram and Narasinghani and Sudhir Kumar (1976) in cowpea.

The genetic changes in quantitative characters induced were realized by the increased genetic variance among progenies in M_2 generation. The extent of induced genotypic variation was as high as 36.00 (at 550 GY gamma rays + 20 mM EMS) for total number of pods per plant (Table 4). Number of pods per cluster showed the least genotypic variation (0.03 at 20 mM EMS, Table 3). Rawlings *et al.* (1958) had stated that the changes induced by the mutagens in chromosomes or genes might have resulted in new and different genotypes in the treated population and this could be the cause for the extended genetic variation observed in M_2 generation. The least genotypic variance observed for pods per cluster also confirmed the dispersion of the mutated alleles in and around the central mean (control).

The broad sense heritability estimates showed maximum value for total number of pods per plant (87.25 per cent at 400 GY gamma rays), number of clusters (81.97 per cent at 0.20 mM EB), seed yield (81.59 per cent at 700 GY gamma rays) and number of seeds per pod (80.31 per cent at 600 GY gamma rays). Estimated genetic gain was as high as 68.43 per cent for total number of pods per plant, at 400 GY gamma rays followed by seed yield per plant (58.39 per cent at 700 GY gamma rays).

In the present study, no consistent relationship could be evidenced between the dose of the mutagens and the observed genetic variability, heritability and genetic advance. There had been comparable mutability following treatment with chemical mutagens with that for ionizing radiations. The higher values of predicted heritability and genetic advance suggested that the mutations might have mostly involved the loci having additive effects. The mean was shifted towards negative direction. However, the enlarged genetic variability, heritability and genetic gain offers a wider scope for further selection. Among the six quantitative traits studied, total number of pods per plant was the highly responding character. Number of pods per cluster was the least responding character. It may be concluded that the culture CO VU 623 of cowpea can be improved upon by selecting for higher total number of pods per plant.

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Isolation and Screening of Phytase Producing Fungi from Soil and Production of Phytase in Submerged and Solid State Fermentation

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One hundred sixty one fungal isolates were obtained from forty soil samples collected from various sites of Meerut region. On screening for phytase activity thirty three isolates were able to grow on medium containing Ca-phytate as sole source of phosphorus. Among 33 isolates, 15 were showed significant zone of hydrolysis. In submerged fermentation using phytase screening broth (PSB) as a production medium, isolate 15E gave highest titer of 31.76 U/mL among the isolates at pH 6.0. while in solid state fermentation using Rice bran and 1% maize flour as substrate a titre of 36.21 U/gDBM was achived at pH 6. The effect of temperature on phytase production among the isolate resulted in an enhancement of approximately 15% from 20 to 40°C. While the production decrease to ~29 - 68 % at 60°C among the isolates. On altering the pH of production medium in submerged fermentation maximum phytase titer in production medium was observed at pH 4.0 and 6.0, with a very slight difference.

Keywords : *Aspergillus niger*, Phytase, Phytic acid, Phytase screening medium, Solid state fermentation, Submerged fermentation.

INTRODUCTION

Enzymes are extremely efficient and highly specific biocatalysis. Microbial enzymes are routinely used in many environment friendly and economic industrial processes (Al Asheh and Duvnjak, 1994; Dvorakova *et al.,* 1997; Harland, and Morris, 1995). Phytase (myo-inositol hexakisphosphate 3-phosphorylase) catalyses the sequential release of phosphate from phytic acid (myo-inositol hexakisphosphate), the organic stored form of phosphorus present in various seeds and grains commonly used as raw materials in foods and feeds (Laumen and Ghisalba, 1994; Pallauf and Rimbach, 1996; Simell, *et al.* 1989; Ullah and Phillippy, 1994; Wodzinski and Ullah, 1996). Phytase reduces the anti-nutritional properties of phytic acid and eutrophication, caused by the excretion of undigested phytic acid by monogastrics because of the lack of adequate levels of phytase in their digestive tracts (Vohra and Satyanarayana 2003; Greiner and Konietzny 2006). These problems could be solved by hydrolysis of phytate using supplemental phytase (Simell *et al.*, 1989). Therefore, phytase has become an important industrial enzyme and is the object of extensive research. By working efficiently on the substrate in the prevailing conditions, supplemental phytase could diminish the anti-nutritive effects of phytic acid and reduce the cost of diets by removing or reducing the need for supplemental inorganic phosphate. In addition, phytase would be an environment friendly product, reducing the amount of phosphorus entering the environment (Haros *et al.* 2001).

MATERIAL AND METHODS

Samples Collection : Forty soil samples were collected in sterile polythene bags from different sites of C.C.S University Campus, Meerut. Sites with high amount of organic contents were preferred for sample collection. Samples were stored at 4°C.

Isolation and Maintenance of Fungal Isolates

Serial soil dilution method : One g of soil sample was suspended in 100 mL of normal saline solution. Shaked well at 200 rpm for 10 min and 1 mL of the suspension was transferred to 9 mL normal saline blank and this procedure was repeated to obtain a dilution of 1/100,000 times. 100 µL of the appropriate dilutions were spreaded on the potato dextrose agar (PDA) plates and incubated at 30 °C for 2-7 days.

Maintenance : After 2 to 7 days of incubation the fungal colonies appeared on plates were transferred to PDA slants

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and incubated for 5 days at 30ºC. The slants with luxuriant growth were stored at 4ºC.

Screening of Phytase Producing Fungi : The qualitative screening was carried out on phytase screening medium (PSM; g/L Calcium phytate 5 g, glucose 15 g, ammonium nitrate 5 g, magnesium sulphate 0.5g, potassium chloride 0.5g, ferrous sulphate 0.01g, manganese sulphate 0.01g, pH- 6.0) (Howson and Davis 1983).

Preparation of Inoculum : Inoculum was prepared from the spore of 7 days old PDA slant culture of fungal isolate. To fully sporulated agar slope culture, 10 mL of sterile normal saline with 0.1% Tween-80 was added. The spores were scrapped using an inoculation needle under strict aseptic conditions. The filtered spore suspension obtained was appropriately diluted to obtain a spore count of ~$1.2X10^5$ spores/mL and used as the inoculum.

Qualitative Screening : Fungi exhibiting the largest zones of clearance on the agar plates were selected and further subjected for qualitative screening.

Quantitative Screening : 100 mL Erlenmeyer flasks containing 20 mL Phytase screening broth were inoculated with 0.5×10^5 - 0.5×10^6 spores/mL and incubated at 30 ºC and 150 rpm for 7 days. 2mL aliquots were aseptically withdrawn from each flask at fix time intervals of 96, 120 and 168 h and centrifuged at 5000g for 15 min. The enzyme activity was calculated using phytic acid as substrate.

Phytase Production in Solid State Fermentation : Five grams of wheat bran with 1% of maize flour were placed in 500mL Erlenmeyer flasks; moistened with 12.5 mL distilled water; autoclaved for 20 min at 121ºC; and cooled to room temperature. The sterilized substrate media were inoculated with one mL of inoculum. The contents were mixed thoroughly and incubated at 30ºC in an incubator. All the experiments were done in duplicate and mean values were reported.

Quantitative Assay for Phytase Activity : Phytase activity was measuring the amount of phosphate released using calcium phytate as a substrate. The reaction mixture comprised of 0.5mL of 0.1M acetate buffer (pH 5.5), 0.5mL of Sodium phytate and 1.0mL crude enzyme. The reaction was carried out at 30ºC for 15 min and the stopped by adding 2.0mL 10% (w/v) TCA. The phosphate released was determined spectrophotometrically.

One unit of Phytase was defined as the amount of enzyme that liberates one µmol of inorganic phosphate mL^{-1} min^{-1} under the assay conditions.

RESULTS AND DISCUSSION

Isolation was carried out from soil samples of C.C.S. University, campus, Meerut, on potato dextrose agar plates. Single colonies were subculture and purified as isolates. In total 161 isolates were obtained.

Screening of Isolates for phytase Production : All the total 161 isolates were tested for phytase production. 33 fungal strains grew well on Ca-phytate agar and develop clear zones around the colonies, among them zone of hydrolysis around the fifteen isolates were significantly high (Fig. 1).

The PSM with 1% maize flour were used for the quantitative screening of phytase production in submerged fermentation resulted in the screening of fifteen isolates for the production of phytase (Kavita *et al.* 2008;). Although enzyme titer of most of the isolates were below 10 UmL^{-1} at pH 6.0 only three isolates showed a titre more then $10UmL^{-1}$. Isolate 15E gave a highest titre of 31.76 UmL^{-1} at pH 6 (Fig. 2).

The visual estimation of the phytate hydrolyzing effect does not distinguish between the phytase activity and the acid phosphatase activity which also precipitates in the total dephosphorylating action of this enzyme group. A significant number of the fungus produces acids which decrease the pH near the colonies. As a result sufficient phosphorus may be released for growth (Vohra and Satyanarayana 2003). In light of the above fact all the isolates showed zone of clearance around the colonies were subjected for quantitative screening.

Filamentous fungi were considered to grow well in solid state fermentation (Pandey 1994) as their mycelia is able to penetrate deep into the solid substrate particles for nutrient uptake, while the bacteria and yeast grow by adhering to the surfaces of the solid substrate particles. In view of the fact that all the fungal isolate showed phytase activity was subjected to solid state fermentation in Erlenmeyer flasks. Rice bran and 1% maize flour were used as substrate. All the isolates were not only able grow well on solid substrate but the extract also showed a significant titre of the enzyme. A highest titre of 36.21 U/GDBM at pH 6.0 were recorded for the isolate no. 15E (Fig. 3).

The effect of temperature on phytase activity at 20 ºC, 40 ºC and 60 ºC was determined. Increasing the temperature from 20ºC - 40ºC resulted in an appreciable increase in phytase activity around ~15%. At 60 ºC phytase activity was found to be reduced by ~29 - 68 % (Fig. 4). The maximum phytase activity was observed at pH 4.0 and pH 6.0, with only a little difference in activity at these two pH Fig. 5 (Man-Jin In, *et al.* 2008).

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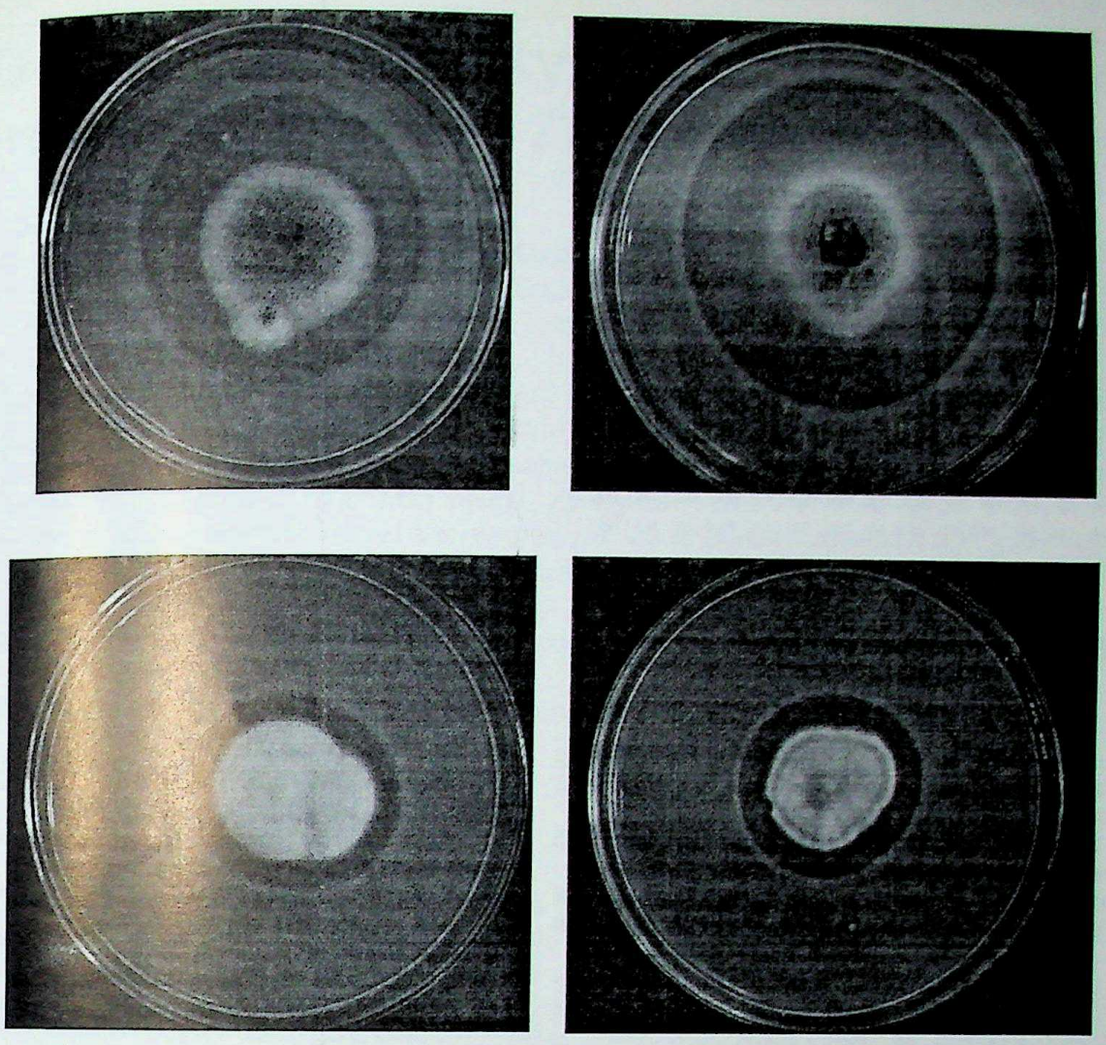

Figure 1. Growth of phytase producing fungal isolates on Ca - phytate agar, clear zone of hydrolysis can be seen around the colonies.

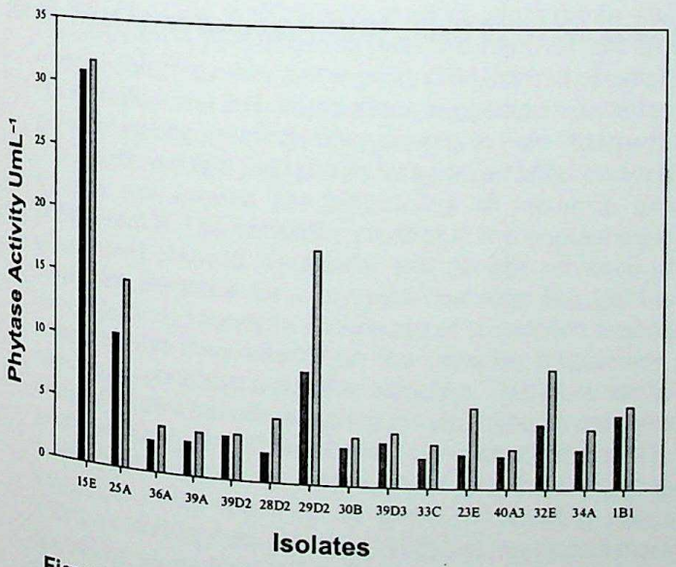

Figure 2. Enzyme titre of isolates in SmF after an incubation of 72 h and 120 h.

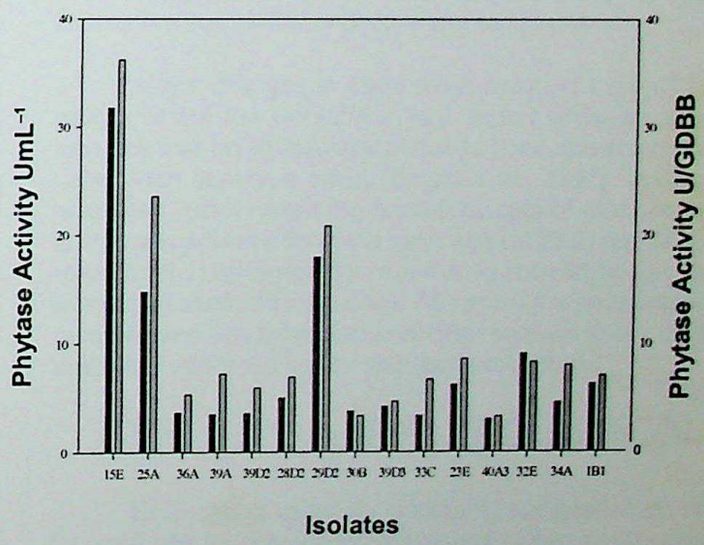

Figure 3. Comparison phytase production in SmF and SSF after 120h of incubation.

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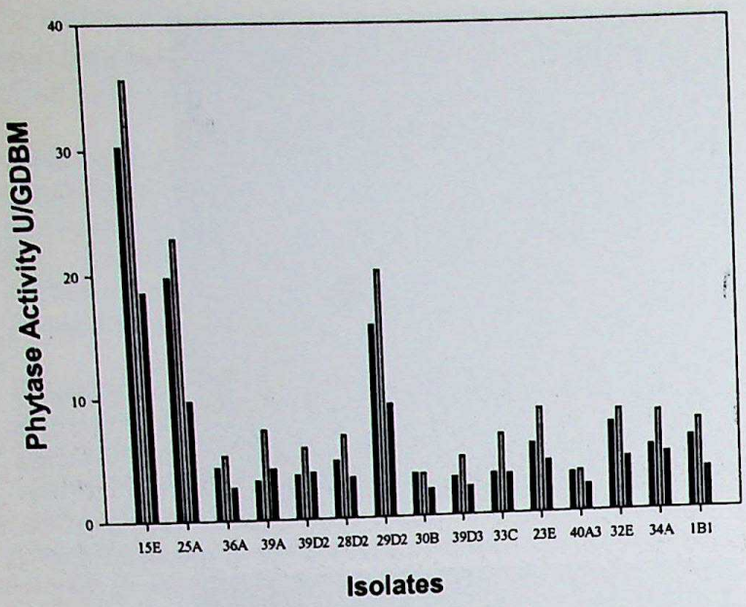

Figure 4. Effect of temperature on phytase activity of isolates

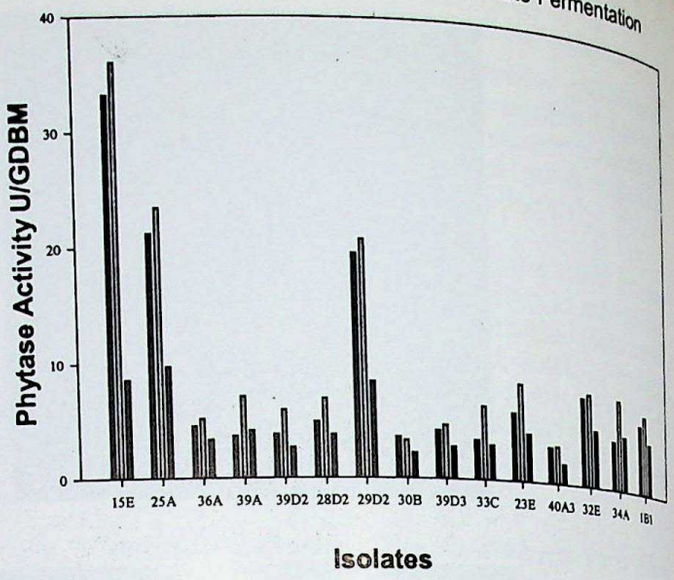

Figure 5. Effect of pH on phytase activity

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Genetic Variability and Correlation Studies in Bread Wheat (*Triticum aestivum* L.)

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The experiment was conducted during 2004 - 05 and 2005 - 06 at research farm of Division of Plant Breeding and Genetics, F.O.A Chatha, SKUAST-Jammu to study the Genetic variability and correlation studies in Bread Wheat (*Triticum aestivum* L.) and the characters studied were days to 50 per cent flowering, plant height, number of tillers/plant, 1000 grain weight, yield/plant which exhibited higher values of variability. The grain yield was positively correlated but plant height was negatively correlated with grain yield similarly grain yield was negatively correlated with harvest index. 1000grain weight, number of grains per ear, tillers per plant, plant height and also for days to maturity. The assessment of extent and nature of different plant characters with grain yield provides breeder to ascertain the real components of yield.

Keywords : Wheat, Genetic Variability, Correlation.

INTRODUCTION

Wheat shares about 20 per cent of the total food calories consumed in the world. It is the second major food crop and occupies premier place among agricultural crops after rice. The assessment of extent and nature of correlations of different plant characters with grain yield provides breeder to ascertain the real components of yield and provide an effective basis of selection. There is sufficient genetic variation in the character through studies of components of variability and heritability. Yield is a unitary character but the end product of interaction of a number of component factors inherent both in the plants as well as in the environment in which plant grows and the improvement in yield depends upon the improvement in these components that make up the yield. (The method of estimation of correlation and their implications suggested that there is no gene for making practical improvements in yield. Adams (1975) has mentioned that yield shoyld be as an architect and breeder should not ignore the principles of balance among components). The variability present in the population in the experiment showed heritability and genetic advance under selection. Selection for a complex character like yield is not very effective. Therefore knowledge of correlation among the characters is their effects on the complex characters and required to construct a suitable selection. Therefore variability, heritability and genetic advance for yield and its components and their analysis with seed yield CV wheat cultivar (1). The method of estimation of correlation and their implications suggested that there is no gene for making practical improvements in yield. Adams, (1975) has mentioned that yield should be as an architect and breeder should not ignore the principles of balance among components.

MATERIAL AND METHODS

The experiment was laid out at the experimental farm of SKUAST-J, Chatha, Jammu. During *rabi* season of 2005-06 to evaluate twenty eight cultivars of wheat. These cultivars were tested in randomized block design with three replications and each treatment consisted of two lines with lines with a row length of 1.5 meters the distance between rows was 22.5 cm apart. (3, 4). This indicates the predominance of additive gene action and thus selection for these characters would be effective. However characters *viz.* tiller per plant and days to maturity showed low heritability of the expression. These traits are mainly governed by several environmental and agronomic factors like soil fertility, moisture and temperature.

Major changes in trend were found in case of spike weight where the heritability was higher while as genetic advance was being the highest due to favorable environment rather than genotype effect (Singh *et al.*, 1997). In case of grains/ear, plant height the heritability was of medium order but genetic advance for these traits was (1179.2) and (2010.9) respectively. Highly significant positively correlation was found with scheduled fertilizer dosage. At harvest the yield data were recorded and was subjected to another analysis for correlation and other variances as per standard procedure.

RESULTS AND DISCUSSION

Estimates of variability, heritability and genetic advance for yield and its component characters of two environments are presented in (Table 1). Among the characters studied days to 50% flowering, plant height, ear length, number of grains per ear, 1000 grain weight and seed yield exhibited wide range

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of variability. Phenotypic and genotypic coefficient of variation for number of tillers per plant, number of ears per plant, plant height and 1000 grain weight were compared to after character namely harvest index. For straw yield, number of grains per ear and grain yield, the coefficient of phenotypic and genotypic variance was high suggesting effectiveness of the phenotypic and genotypic selection. Paroda and Joshi (1970) reported that number of ears per plant; grains per ear and grain weight were the major yield components. Higher heritability of yield (326.71) per cent was recorded for spike weight (Table-2) followed by number of tillers per plant, grains per ear and days to maturity. It was negatively correlated for plant height, harvest index, 1000 grain weight, days to 50 % flowering. The phenotypic correlation with days to 50 % flowering was

Table 1 : Analysis of variance for different characters under normal (E1) and stress (E2) environments.

Characters	Source	df	Y1/E1	Y1/E2	Y2/E1	Y2/E2
No. of days to 50 % flowering	Replication	2	00.96	74.87	140.63	155.83
	Genotype	27	04.58**	789.34**	65.71**	75.82**
	Error	54	00.32	19.53	34.31	33.05
Days to maturity	Replication	2	10.21	20.46	654.14	66.71
	Genotype	27	30.67**	50.11**	9.89**	40.16**
	Error	54	06.11	9.43	8.03	7.71
Plant height (cm)	Replication	2	12.65	8.16	00.41	74.89
	Genotype	27	67.45**	84.71**	70.62	789.35**
	Error	54	4.96	05.18	01.47	19.54
No. of tillers/plant	Replication	2	00.04	00.13	77.58	03.33
	Genotype	27	10.61**	08.40**	37.93**	03.69**
	Error	54	00.45	00.87	18.36	01.19
Ear length (cm)	Replication	2	00.11	00.19	01.76	02.67
	Genotype	27	03.84**	05.76**	03.66**	08.46**
	Error	54	02.11	03.76	01.15	02.21
No. of grain/ear	Replication	2	01.29	00.19	00.14	01.30
	Genotype	27	49.48**	33.16**	134.12**	82.24**
	Error	54	06.62	03.11	10.57	01.16
Biomass/plant (g)	Replication	2	110.00	87.10	80.10	66.18
	Genotype	27	166.42**	76.40**	32.35**	40.14**
	Error	54	07.27	5.14	7.10	8.11
Grain yield/ plant (g)	Replication	2	0.94	00.85	46.43	00.08
	Genotype	27	08.25**	10.50**	650.22**	20.23**
	Error	54	00.16	00.02	84.08	0.014

			Mean square			
Characters	**Source**	**df**	**Y1/E1**	**Y1/E2**	**Y2/E1**	**Y2/E2**
Harvest index	Replication	2	00.05	00.15	49.05	30.31
	Genotype	27	24.03*	06.08*	650.23**	46.44**
	Error	54	04.05	01.16	4.97	02.11
1000 Grain weight (g)	Replication	2	00.51	00.16	00.41	1.38
	Genotype	27	30.23**	20.18**	70.62**	66.89**
	Error	54	21.05	14.14	01.47	1.50
Relative water content of leaf	Replication	2	79.10	87.15	67.15	77.11
	Genotype	27	08.10**	10.09**	04.14*	7.17**
	Error	54	00.15	00.11	0.11	0.14
Relative water loss of leaf	Replication	2	87.11	79.11	77.60	87.20
	Genotype	27	11.10**	08.11*	7.12*	5.15*
	Error	54	02.10	1.14	0.11	1.10
Drought susceptibility index	Replication	2	0.11	0.10	0.11	0.18
	Genotype	27	0.15	0.17	0.14	0.09
	Error	54	0.02	0.10	0.07	0.03
Proline content	Replication	2	0.02	0.10	0.09	0.07
	Genotype	27	0.44	0.32	0.18	0.09*
	Error	54	0.02	0.12	0.13	0.05

**,* Significant at 5 and 1 per cent level, respectively

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Table 2 : Different plant characters showing range, mean, CV, GCV, PCV, heritability and genetic advance.

Character	Range	Mean/SE	C.V%	G.C.V.	P.C.V.	h_2b heritability	Genetic advance	GA% mean
Days to 50% flowering	95-102	0.599	0.36	0.38	0.286	178.397	304.521	105.28
No.of Tillers/plant	8-27	3.49	0.023	17.286	33.76	26.2106	269.34	1823
Plant height	89-128	14.812	17.04	9.663	10.181	90.066	2010.965	1889.07
Straw yield	11-32	3.7049	0.1178	0.0938	-0.0665	215.4344	1133.837	29.446
Spike wt.	3-27	3.011	6.3504	11.4786	6.3504	326.71	3737.2	6435.53
Days to maturity	128-143	2.3141	0.6932	0.1926	0.7194	7.1656	43.42	10.62
Grains/ear	15-29	2.6552	13.796	27.2248	30.5208	79.5789	1179.16	50.0028
Grain yield/plant	3-90	7.8623	15.953	22.2327	27.3646	0.66	22.458	37.4259
1000gr.wt.	33-52	0.9896	1.059	23.05	21.58	106.81	1022.14	737.1

Table 3 : Correlation between grain yield and various drought related traits.

Character	Correlation
Drought susceptibility Index Vs Yield(S)	0.5809**
Drought susceptibility Index Vs Yield(NS)	0.3727**
Germination Stress Index Vs Yield(S)	0.1132**
Germination Stress Index Vs Yield(NS)	0.0525*
Relative water content Vs Yield(S)	0.1806*
Relative water content Vs Yield(NS)	0.114*
Relative water Loss Vs Yield(S)	–0.1759**
Relative water Loss Vs Yield(NS)	–0.1020**
Proline Vs Yield(S)	–0.0280*
Proline Vs Yield(NS)	0.082*
Seed Died Vs Yield(S)	0.1182**
Seed Died Vs Yield(NS)	–0.153**

**,* Significant at 5 and 1 per cent level, respectively

negatively correlated with grain yield, 1000 grain weigh was negatively correlated with grain yield, and there by suggesting that improvement of any of these characters may simultaneously improve and other except 1000 grain weight significantly and positively correlated but grain yield negatively correlated with harvest index and 1000 grain weight, also number of tillers per plant simultaneously both grain and straw yield were positively correlated with grain yield. The phenotypic correlation with number of tillers and days to 50 % flowering was negatively correlated with ear length, 1000 grain weight, harvest index and days to 50 % flowering, straw weight (215.4) per cent and days to 50 % flowering, plant height, 90.1 grains per ear (79.5 %), tillers per plant 26.2 % and heritability for other characters was of medium range.

This indicates the predominance of additive gene action and the selection for these characters involved be effective the low heritability in grain yield, days to maturity and tillers per plant and the expression of these traits are mainly governed by several agronomic factors and environment factors like soil fertility, moisture and temperature, the major change in trend was found in case of spike weight were the heritability was found higher while as genetic advance was also higher and it was suggested favorable environmental influence rather than genotypic effect (5). In case of grain yield the heritability was of lower range.

Highly positive correlation (Table-3) of days to maturity and grain weight found significantly positive correlation for plant height, grains per ear, harvest index, 1000 grain weight. While as positive correlation was observed in plant height and it has positively correlated with grain yield. However, low positive correlation of days to maturity, number of tillers per plant, grains per ear. These results are in agreement with those of Singh *et al* . (1997). However days to maturity *viz.* days to 50 % flowering was negatively correlated also days to maturity plant height was negatively correlated and significant 1000 grain weight, ear length, spike weight, straw weight, grain weight, grains per ear, harvest index and number of tillers per plant, plant height were positively correlated with days to 50 % flowering. Plant height with spike weight, ear length and grain yield further suggested the limitation of increasing straw yield without reduction in harvest index. The correlation between yield and some quantitative traits in bread wheat and reported that main yield component was 1000 grain weight which accounted for 20.2 per cent of variation in yield and number of spikelets per ear accounted for 5 per cent of variation.

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Genetic Variability for Quantitative Characters in Sweet corn (*Zea mays Saccharata*)

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Twenty five sweet-corn genotypes were evaluated for eleven quantitative characters in mid hills of Himachal Pradesh. A significant difference among the entries for almost all the studied characters was recorded. The characters 100-grains weight, number of seeds per row and grain yield per plot were the most important parameters to be considered in parents for effective selection before initiating actual crossing programme, since these attributes exhibited high variability, heritability combined with high genetic advance as per cent of mean. Eight superior sweet corn lines with desirable characters were identified for future use in breeding programmers.

Keywords : Genetic advance, Genetic variability, Sweet-corn.

INTRODUCTION

Sweet-corn is a highly valuable and industrially useful type of maize used as raw green cob or after preservation for table purposes. The genetic composition of sweet corn kernels differ from the corn in only one recessive gene *su1* in contributions with few modifiers, whereas in super sweet corn *su1* and *su2* genes prevent the conversion of sugars into starch in the endosperm. Sweet corn differs from dent corn by one recessive gene *'su'* on 4th chromosome which prevents the conversion of part of sugars into starch. It has been reported to be a mutant from field corn (Kempton, 1928; Linder storm, 1929; Smith, 1955). Madhuri a sweet corn variety first of its kind in the country was released from Maize Research Station, Amberpet, Hyderabad during 1990. This variety has become popular in many parts of northern and southern states of India. It contains about 9% total sugars in dried seed while up to 33% on milky or fresh grain basis. Similarly a sweet corn composite (Bajaura Sweet corn) has been released for cultivation in hills by HAREC, Bajaura (H.P.). Improvement of sweet corn yields, while retaining quality is one of the major challenge facing the sweet corn breeders (Hunsperger and Davis, 1987). Hence, an efforts was made to study the germplasm for different characters and to identify superior sweet corn genotypes that could be further used in future crossing programme to develop high yielding varieties and hybrids.

MATERIAL AND METHODS

The germplasm used in the present study consisted of 25 sweet corn collections laid out in randomized block design with three replications. Each genotype was grown in a single row of 5m. length, spaced 75 cm apart with a 20 cm distance between plants within the row. Five competitive plants were selected at random in each entry for recording the observations on plant height (cm), ear height (cm), ear length (cm), ear girth (cm), 100 grain weight (g), number of seed rows per ear and number of seeds per row while data relating to days to 50 per cent silking, days to 50 per cent maturity and grain yield (Kg) were recorded on whole plot basis. The genetic parameters *viz.*, genotypic and phenotypic coefficient of variation (Burton, 1952), heritability in broad sense and genetic advance as per cent of mean (Johnson *et al.*, 1955) were studied for all the characters.

RESULTS AND DISCUSSION

The entire success of plant breeding programme in any crop largely depends on the range of variability owing to genetic and non-genetic causes, present in that crop (Vavilov, 1951). Hence, the present investigation was conducted to assess the genotypic variability in sweet corn germplasm for 11 characters to identify superior genotypes. Analysis of variance indicated significant difference between the genotypes in respect of all the morphological, quantitative and qualitative parameters (Table-1). The characters *viz.*, grain yield per plot (29.54% and 29.45%), 100 grain weight (26.51% and 26.44%) and number of seeds per row (23.73% and 22.50%) were observed to possess high PCV and GCV estimates (Table-2), indicating that, the selection based on these characters would facilitate, easily, the successful isolation of desirable genotypes. Similar findings were earlier reported for these characters in sweet corn by Satyanarayana and Sai Kumar 1995 and 1996), Mani

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and Bisht (1996), Cartea *et al.*, (1996), Hatch Kiss *et al.*, (1997) Devadas Ramgopal (1999) and Satyanaraina *et al.* (2003).

Wide difference between PCV and GCV estimates (Table-2) were observed for ear girth (1.20), revealing greater role of environment interaction with the genetic factors in the expression of this traits, where as plant height, ear height, 100 grain weight, days to 50 per cent maturity, days to 50 per cent silking and days to 50 per cent tasseling exhibited lower difference indicating more influence of genetic factors in determining variability. These observations were also supported by earliler studies of Satyanarayana and Sai Kumar (1995, 2003) and Devadas Ramgopal (1999).

The GCV alone is not sufficient for the determination of amount of heritable variation. Burton (1952) suggested that GCV together with the heritability estimations would give the best picture of the extent of advance to be expected by selection. In the present investigation, high heritability was registered for ear height (99.45%) followed by plant height (98.62%) and 100 grain weight (98.55%), which is in consonance, with the results of Satyanarayana and Sai Kumar (1996) and Devadas Ramgopal (1999). Low heritability estimates were recorded for ear girth (83.25%) and ear length (85.49%). Devadas Ramgopal (1999) also recorded this type of observations. Grain yield per plot, sugar content, 100 grain weight and number of seeds per row showed high GCV estimates, coupled with high heritability there by pointing to the improvement of these characters through simple per se selection and using them in hybrid breeding programme for development of heterotic hybrids.

The heritability estimates indicate only the effectiveness with which selection of genotype can be based on phenotypic performance but fail to indicate the real progress and on the other hand high heritability could be also due to lower influence of environment on genotypes. Therefore, high heritability need not always accomplished by genetic progress. However, high heritability estimates along with high genetic gain render the selection effective (Johnson *et al.*, 1955). In this study the genetic advance as per cent of mean was maximum for grain yield per plot (55.55%), followed, 100 grain weight (54.30%) and number of seeds per row (52.90%) along with high heritability estimates. This situation indicates that the genotypic variance for these characters is probably owing to their high additive gene effects (Johnson *et al.*, 1955). This also confirms the earlier findings of Singh and Sharma (1970), Devadas Ramgopal (1999). High heritability combined with lowest genetic advance as per cent of mean was noted for days to 50 per cent maturity (8.99%), plant height (12.87%), days to 50 per cent silking (16.90%) and days to 50 per cent tasselling (15.41%) revealing little scope for improving these characters through selection and this may be attributed to the non-additive gene effects on these traits (Johnson *et al.*, 1955). Similar types of findings were also reported by Reddy and Agarwal (1992) and Devadas Ramgopal (1999) and Satyanaraina *et al.* (2003).

In brief it can be stated that the traits *viz.*, 100-grain weight, number of seeds per row, and grain yield per plot which exhibited high variability, heritability and genetic advance, are the most important characters to be considered for effective selection of parental lines to develop a high yielding sweet corn variety or hybrid. Top eight sweet corn germplasm (*viz.*,PSS-1, SSK,Sweet-2,HSS,Mas Madhu,MSS-1,MSS-101 and PSS-3) (Table-3) with desirable characters were identified in the present study and these lines will be utilized in future breeding programmmes to devlop high yielding sweet corn variety or hybrid.

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Table 1 : ANOVA for 11 characters in sweet corn genotypes.

Characters	Treatment M.S.S.	C.D.	C.V.
Days to 50% tasselling	27.49**	1.82	1.66
Days to 50% silking	28.00**	1.62	1.42
Days to 50% maturity	41.55**	2.10	1.20
Plant height (cm)	198.66**	1.45	0.61
Ear height (cm)	160.56**	1.23	1.20
Ear length (cm)	5.98**	1.92	3.66
Ear girth (cm)	2.15**	1.18	4.95
100grain weight (g)	46.66**	0.92	1.85
Number of grain per row	39.25**	1.52	3.41
Number of grain per cob	6.84**	1.45	4.51
Grain yield per plot (Kg)	0.150**	0.15	7.95

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Table 2 : Estimates of genetic parameters for 11 characters in sweet corn genotypes.

Character	Grand mean	Range		PCV (%)	GCV (%)	Heritability (%)	Genetic advance	GA as % over mean
		Min	Max					
Days to 50% tasselling	48.22	40.00	56.60	7.66	7.69	94.68	7.43	15.41
Days to 50% silking	51.73	46.20	58.90	7.43	7.50	95.76	7.81	16.90
Days to 50% maturity	104.12	94.80	112.20	4.54	4.44	96.10	9.36	8.99
Plant height (cm)	155.56	135.70	174.10	6.40	6.58	98.62	20.02	12.87
Ear height (cm)	46.22	36.70	74.60	18.12	18.09	99.45	17.55	37.97
Ear length (cm)	11.49	7.66	16.40	15.50	14.40	85.49	3.40	29.59
Ear girth (cm)	7.93	6.25	11.20	13.64	12.44	83.25	1.90	23.95
100 grain weight (g)	16.83	9.85	26.35	26.51	26.44	98.55	9.14	54.30
Number of grain per row	17.20	8.60	28.11	23.73	22.50	96.18	9.10	52.90
Number of grain rows per cob	12.06	10.22	17.25	14.45	14.95	90.12	3.85	31.92
Grain yield per plot (Kg)	0.81	0.24	1.66	29.54	29.45	95.65	0.45	55.55

Table 3 : Mean data of eight superior sweet corn lines characters.

Sweet corn lines	100 grain weight (g)	Number of seeds/row	Grain yield / plot(Kg)
PSS-1	24.50	13.80	1.45
SSK	25.20	25.50	1.65
Sweet-2	22.15	18.00	1.32
HSS	16.18	24.30	0.99
Mas Madhu	18.58	25.00	0.98
MSS-1	17.85	27.30	1.54
MSS-101	24.35	28.10	1.45
PSS-3	19.85	22.40	1.68

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Plasticity of an Alkaline Tolerant Mulberry Genotype under Various Growing Environments for Sustainable Leaf Production

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The present study subjected RC-2, an alkalinity tolerant mulberry genotype, to different stress conditions like low input (fertilizer), soil moisture stress, alkalinity and salinity along with a control genotype K-2 maintained at pH 7.0, with recommended dosage of nutrients (NPK) and irrigation. Significant variability was recorded between the genotypes in respect of leaf yield response index [LYRI], stress resistance index [SRI] and varietal score [VS]. The results indicated a high degree of plasticity in RC-2 that would assure sustained leaf production in alkaline and semi-irrigated tracts. The genotype could be recommended for cultivation by the marginal and small farmers with alkali-affected soils for economic utilization of the problematic soils through sericulture.

Keywords : Plasticity, Alkalinity Tolerance, Mulberry.

INTRODUCTION

Growing demand for silk in the domestic and international markets offers great opportunities for an agrarian country like India, that has a tradition in silk production. While increased productivity per unit area has significantly contributed towards production, in view of the competition for land and resources from other food and commercial crops, it is imperative to develop strategies for utilizing problematic soils including alkali affected soils. It is estimated that nearly 4.5 million hectares of potential agricultural land in India are affected by soil alkali conditions. Larger areas under the semi-irrigated conditions exhibit alkalinity due to poor rainfall and scarcity of irrigation water. These soils contain excessive concentrations of exchangeable carbonates or bicarbonates of sodium that usually exceeds 30% of the soils cation exchangeable capacity and high pH, which affect plant survival and growth due to physiological drought conditions and nutritional deficiencies. Utilization of problematic soils is best achieved by growing tolerant plant species (Epstain, 1985; Epstain and Rains, 1987; Ashraf and Mc Neilly, 1988). Although soil amendment and management is one of the feasible means, genetic improvement of crops towards tolerance to stress is more effective, less costly, non-polluting and longer lasting (Epstain *et al.*, 1980; Downtown, 1984).

Many studies have been conducted to identify the salt tolerant mulberry genotypes by screening under coastal saline soils (Agastian and Vivekanandan, 1997; Chakraborty *et al.*, 2000) and under induced salinity conditions (Shaik Mohamed Anas and Vivekanandan, 1999; Mogili *et al.*, 1998, 2002; Prakash *et al.*, 1998; Sarkar *et al.*, 2000). Naidu *et al.*, (1999) reported variability in reaction to alkalinity stress among tree species when screened under natural stress conditions.

The present study subjected RC-2, a mulberry genotype tolerant to alkalinity, to different stress conditions like low input (fertilizer), soil moisture, alkalinity and salinity.

MATERIAL AND METHODS

The study was carried out under controlled conditions using pot culture technique. The alkaline tolerant mulberry genotype, RC-2 was considered for the study along with K-2, a variety popular under semi-irrigated conditions, as check. Well-rooted saplings of the two genotypes were planted in pots lined with polyethylene sheets to provide full effect of the treatments under simulated conditions to study the plasticity of genotypes under different conditions. The different conditions were treated as environments.

The genotypes were put under fertilizer stress (50% & 25% of the recommended dose), soil moisture stress (irrigation once in 3 & 7 days), salinity stress (Ec- 4.0 & 7.0 $mmho^{-cm}$) apart from alkalinity stress (pH 8.5 & 10.0). The controls were maintained with recommended dosage of fertilizers *i.e.*, N:P:K @ 300:120:120 Kg/ha/yr in 5 equal splits, pH 7.00 and Ec 0.8 $mmho^{-cm}$.

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Nine different conditions comprising of optimal and eight different conditions were simulated.

E 1- Optimal-water daily (7/7), full dose of fertilizers (F)
E 2- Water daily, 50% of recommended dose of fertilizers (F/2)
E 3- Water daily, 25% of recommended dose of fertilizers (F/4)
E 4- Water once in 3 days (1/3), full dose of fertilizers
E 5- Water once a week (1/7), full dose of fertilizers
E 6- Water daily, full dose of fertilizers, pH 8.50
E 7- Water daily, full dose of fertilizers, pH 10.0
E 8- Water daily, full dose of fertilizers, Ec 4.0 $mmho^{-cm}$
E 9- Water daily, full dose of fertilizers, Ec 7.0 $mmho^{-cm}$

The stress levels were induced by adopting different frequency of irrigation; fertilizer inputs in the form of Ammonium sulfate (20% N), Single super phosphate (16% P) and Murate of potash (60% K) to achieve 50% and 25% of recommended dosage of N:P:K (300:120:120); adding Sodium carbonate and Calcium carbonate to induce alkalinity; adding Sodium chloride and Calcium carbonate to induce salinity. Soil samples were collected periodically from each pot where different salinity and alkalinity levels were induced and analyzed after each harvest. The data indicated slight increase in pH and Exchangeable Sodium Percentage (ESP) and Sodium Adsorption Ratio (SAR). However, pH, ESP and SAR were within normal range of variation and did not affect the actual stress conditions induced. The experiment was conducted in randomized design with three replications, considering individual pots as replications. Each ordered replicate was arranged compactly with a guard row around each replication. Pots were shifted at regular intervals and data recorded each time during harvest.

The experiment was conducted for one year after establishment of saplings in pots. Leaves were harvested at intervals of 70 days and the data of five leaf harvests were recorded. Average leaf yield was considered for further analysis.

Plastic response of genotypes was measured by one-way and two-way ANOVA. Tolerance indices for different stress environments were determined following Rana (1986) and Maloo (1993) with slight modifications :

Leaf yield response index [LYRI]

$$LYRI = \frac{\text{Leaf yield of a variety (average of all stress environments)}}{\text{Mean yield of all varities under stress environments}}$$

Stress resistance index [SRI]

$$SRI = \frac{\text{Leaf yield of a variety (average of all stress environments)}}{\text{Leaf yield of the variety under non-stress environment}}$$

Varietal score [VS] = LYRI x SRI

RESULTS AND DISCUSSION

The mean values for leaf yield at different growth environments are presented in Table 1. Significant reduction was recorded in leaf yield of both the genotypes in response to different stress factors and also in the yield between the genotypes under a particular environment. The leaf yield of the test genotype RC-2 significantly reduced in all the treatments (E2 to E9) compared to the control (E1). The reduction in leaf yield was not similar in all the treatments. Under moderate stress condition the leaf yield was found to vary from 82.28% in E4 to 71.66% in E2, in comparison to the yield in E1. The yield level varied from 70.63% in E9 to 44.16% in E7. There was no significant difference between the two fertilizer doses E2 and E3 and salinity treatments E8 and E9 Maximum reduction was observed in E7. However, in all the treatments RC-2 was found superior to K-2 in respect of leaf yield, with the largest positive yield variance in case of mild soil moisture stress (87.60%), followed by mild alkalinity (51.87%) and severe salinity (50.24%). The variance was 47.96% in case of control maintained under non- stress conditions.

The leaf yield was dependent on both genotype and environments. Genotype x environment interaction was also found to be significant. The genotype term was found significant in comparison to K 2. Both environment and G x E terms were found to be significant indicating the plastic response of the genotypes and differences in their response (Table 2).

The tolerance indices measured by using leaf yield under stress and control conditions indicated clear differences between the two genotypes (Table 3). Leaf yield response index (LYRI) was found to be very high in RC-2 in comparison to K-2. Stress resistant index (SRI) was found to be nearly the same in both the genotypes. Varietal score (VS), which measures the tolerance level, was found to be very high in RC-2 under all the growing conditions. Rana (1986) and Singh (1991) critically analyzed the selection criteria for salt tolerance in crop plants and concluded that the tolerant genotype shows higher rankings for leaf yield response, stress resistance indices and varietal scores.

The results indicated a high degree of plasticity in RC-2 that would assure sustained leaf production in alkaline and semi-irrigated tracts. The genotype could be recommended for cultivation by the marginal and small farmers with alkali-affected soils for economic utilization of the problematic soils through sericulture. This will not only increase the silk production but also will improve the economy of the poor farmers in those areas.

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Table 1 : Mean values for leaf yield (g/plant) under different growing environments in two mulberry genotypes*.

Sl. No.	Environment	Mulberry genotype		Increase over check (%)
		K-2 (Check)	RC-2	
1.	E1-Non-stress	95.16	140.80	47.96
2.	**Fertilizer stress**			
	E2-Mild (50% dose)	75.96	100.90	32.83
	E3-Severe (25% dose)	65.26	89.26	36.77
3.	**Soil moisture stress**			
	E4-Mild (once in 3 days)	61.76	115.86	87.60
	E5-Severe (once in 7 days)	50.40	67.96	34.86
4.	**Alkalinity stress**			
	E6-Mild (pH- 8.5)	71.73	108.94	51.87
	E7-Severe (pH-10)	41.91	62.19	48.39
5.	**Salinity stress**			
	E8-Mild (Ec-4.0 mmho^{-cm})	82.50	111.23	34.82
	E9-Severe (Ec-7.0 mmho^{-cm})	66.20	99.46	50.24
	C. D. at 5%	10.32	14.44	–

* Results of one-way ANOVA

Table 2 : Comparison of plastic response in leaf yield between two mulberry genotypes grown in different environments*.

Genotypes compared	Mean squares	F-test indication
RC-2 *Vs* K-2	Genotype : 262.37	Significant at 1% level
	Environment : 44.62	Significant at 1% level
	Genotype x Environment : 4.23	Significant at 1% level

C.D. value for interaction between Genotype x Environment : 11.93

*Results of two-way ANOVA

Table 3 : Indices for measuring tolerance in two mulberry genotypes under different stress conditions.

Stress conditions	Mulberry genotypes	
	K-2	RC-2
	Leaf yield response index [LYRI]	
Fertilizer stress	0.85	1.15
Soil moisture stress	0.75	1.24
Alkalinity stress	0.80	1.20
Salinity stress	0.83	1.17
	Stress resistance index [SRI]	
Fertilizer stress	0.74	0.67
Soil moisture stress	0.59	0.65
Alkalinity stress	0.60	0.61
Salinity stress	0.78	0.74
	Varietal score [VS]	
Fertilizer stress	0.63	0.77
Soil moisture stress	0.44	0.73
Alkalinity stress	0.48	0.73
Salinity stress	0.65	0.87

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Sequence Analysis of Rice Chromosome 4

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Rice is an excellent system for plant genomics as it represents a modest size genome of 430 Mb. It feeds more than half the population of the world. Beyond its importance as the world's premier crop, rice is also an excellent model plant for genomics. EST provides a snapshot of the transcriptome at a particular moment. Annotation describes the genome by identifying various sites and segments along the sequence involved in the functioning of the genome. The main objective is definitely the positioning of the genes and the elements related to the functioning of these genes or their products. A careful analysis of this sequence data can provide significant additional functional, structural and evolutionary information. It can be stated that ESTs provide an inexpensive and rapid approach to gene discovery, the characterization of gene expression and regulation, the identification of gene function and in the study of alternative splicing.

INTRODUCTION

Expressed Sequence Tags (ESTs) are currently the most widely sequenced nucleotide commodity in the terms of number of sequences and total nucleotide count. These provide a robust sequence resource that can be used for genome annotation, gene discovery and comparative genomics (Rudd, 2003). The concept of using cDNA as a route to expedited gene discovery was first discovered in early 1980s (Putney *et al.*, 1983). With the widespread studies being performed on rice, a number of genetic markers have been mapped aiding in the development of a comprehensive genetic and physical map of rice (Chen *et al.* 2002). The vast amount of structural and functional data of the rice genome generated earlier (Sasaki 1998; Sasaki and Burr 2000) provided a strong backbone for its sequencing efforts (Delseny *et al.* 2001; Sasaki 2002). Today, however, genome annotation is performed using a combination of experimental and computational methods, with the latter forming a significant part of the expanding field of bioinformatics. Many bioinformatics resources are now available to researchers around the world through the World Wide Web (WWW). A recently developed informatics resource specifically dedicated to rice is the MIPS Rice (*Oryza sativa*) database or the MOsDB (http://mips.gsf.de/proj/rice; Karlowski *et al.* 2003). On chromosome-4, 47.7% of the genes were predicted correctly in the *indica* sequence draft, while rest were either partial (38.3 %) or absent (13.85 %). This is probably because *indica* draft sequence has comparatively more gaps, which would significantly affect the process of annotation (Feng *et al.* 2002). In cases where the genome of a species is already available, a spliced alignment of such consensus sequences can lead to identification of the locations of the respective genes, this is a more efficient alternative to direct spliced alignment of the ESTs to the genome, because of the latter's redundancy (Coward *et al.*, 2002).

MATERIAL AND METHODS

Collection of whole sequence of chromosomes-4 : We can download the sequences from the Gramene database (http://www.gramene.org) of Rice species *Oryza sativa* ssp. japonica various other sites like Genome project from NCBI. These sequences are the input data in our EST analyzer program (GenMark).

GenMark-E - This program is used for Gene Prediction in EST and cDNA. GeneMark-E (http://opal.biology.gatech.edu/GeneMark/) program determine the protein-coding potential of a DNA sequence (within a sliding window) by using species specific parameters of the Markov models of coding and non-coding regions. GeneMark is documented as the most accurate prokaryotic gene finder.

Steps for the annotation of EST sequences of chromosomes 4 of Rice:

1. First of all we can download the sequence from the Gramene database or the map viewer of Genome project of NCBI of *Oryza sativa.*
2. Chromosome 4 is of 45KB.
3. Cutting of these large sequences into small pieces by using parameters while downloading the sequences of all chromosomes.
4. Paste these sequences one by one into the program GenMark-E.
5. Click the species *O. sativa* and in the text output options click the nucleotide transcripts regions and protein translations regions. Start GenMark.
6. Analysis of ESTs has been done by above process.
7. BlastX searching of these contigs against the nr database at the NCBI.
8. Assigning functions to the contigs via the BlastX searches if contigs finding any matches.

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Table 1 : Predicted Genes.

Predicted genes	Annotation info	No. of EST
Os04g0100300	Actin-binding FH2 domain	33
Os04g0101300	MRG family protein	6
Os04g0101400	Cytochrome P450 family protein	111
Os04g0101700	Engulfment and cell motility, ELM domain	15
Os04g0102500	Phosphoglycerate mutase-like protein	57
Os04g0102600	Splicing factor 3B subunit 2	54
Os04g0102700	N-acylethanolamine amidohydrolase	47
Os04g0103100	Glycosyl transferase, family 43 protein	12
Os04g0103200	Proteasome component region PCI domain	165
Os04g0103300	Conserved hypothetical protein	208
Os04g0103500	Serine/threonine protein kinase family protein	10
Os04g0103700	Protein kinase domain containing protein	18
Os04g0103800	Conserved hypothetical protein	2
Os04g0103900	Chalcone synthase (EC 2.3.1.74)	2
Os04g0104900	Caffeic acid 3-O-methyltransferase	18
Os04g0105000	Zn-finger,C-x8-C-x5-C-x3-H type domain	45
Os04g0105100	Zn-finger, RING domain	73
Os04g0105200	Conserved hypothetical protein	60
Os04g0105300	Protein of unknown function DUF1295 family protein	10
Os04g0105400	Conserved hypothetical protein	27
Os04g0105500	Uridylate kinase	35
Os04g0105700	Conserved hypothetical protein	70
Os04g0106000	Plant invertase/pectin methylesterase inhibitor domain	4
Os04g0106300	Arginase	67
Os04g0106400	D-isomer specific 2-hydroxyacid dehydrogenase	6
Os04g0107200	D-isomer specific 2-hydroxyacid dehydrogenase	6
Os04g0107300	D-isomer specific 2-hydroxyacid dehydrogenase	1
Os04g0107500	D-isomer specific 2-hydroxyacid dehydrogenase	14
Os04g0107600	Arginine decarboxylase (EC 4.1.1.19) (ARGDC)(ADC)	38
Os04g0107700	Peptidase CIA, papain family protein	6
Os04g0107900	Heat shock protein 81-1(HSP81-1)(Heat shock protein83)	10
Os04g0108300	Protein of unknown function DUF177 domain	22
Os04g0109100	Protein kinase domain containing protein	2
Os04g0109200	Conserved hypothetical protein	2
Os04g0109500	Concanavalin A-like lectin/glucanase domain	10
Os04g0110100	Concanavalin A-like lectin/glucanase domain	4
Os04g0110200	Conserved hypothetical protein	7
Os04g0110300	SWIRM domain containing protein	17
Os04g0110500	Protein prenyltransferase domain containing protein	12
Os04g0110600	Fus-prov protein-contains InterPro domain(s): IPR001876	30
Os04g0111200	ATP sulfurylase precursor (Sulfate adenylytransferase)	13
Os04g0111300	Hypothetical protein	1
Os04g0111500	Conserved hypothetical protein	56
Os04g0111900	Virgal	56
Os04g0112000	Conserved hypothetical protein	2

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Os04g0112300	Eukaryotic initiation factor 3, gamma subunit	35
Os04g0115200	Conserved hypothetical protein	57
Os04g0115400	D111/G-patch domain containing protein	30
Os04g0115500	Zn-finger, C2H2 type domain containing protein	10
Os04g0115700	Hypothetical protein	63
Os04g0115900	Conserved hypothetical protein	2
Os04g0116200	Conserved hypothetical protein	13
Os04g0116600	Short-chain dehydrogenase/reductase SDR family protein	10
Os04g0116800	Naringenin-chalcone synthase family protein	5
Os04g0116900	Conserved hypothetical protein	7
Os04g0117100	Conserved hypothetical protein	117
Os04g0117200	Spectrin repeat containing protein	18
Os04g0117300	SEC7-like domain containing protein	5
Os04g0117600	Dihydrouridine synthase, DuS family protein	29
Os04g0117800	Chloroplast Toc64-1	93
Os04g0117900	Toc64	93
Os04g0118100	Amidase family protein	353
Os04g0118400	Elongation factor EF-2	51
Os04g0118600	Hypothetical protein	4
Os04g0118700	Protein prenyltransferase domain	4
Os04g0118900	Arginine/serine-rich splicing factor RSP31	26
Os04g0119000	Conserved hypothetical protein	20
Os04g0119200	Conserved hypothetical protein	4
Os04g0119300	Conserved hypothetical protein	49
Os04g0119400	Pyruvate dehydrogenase E1 alpha subunit	224
Os04g0119500	Membrane related protein-like	156
Os04g0119800	Conserved hypothetical protein	3
Os04g0120100	Conserved hypothetical protein	2
Os04g0121100	Peptidase S8 and S53,subtilisin,kexin,sedolisin domain	2
Os04g0121300	Subtilase	7
Os04g0122000	Leucine-rich repeat, cysteine-containing type	67
Os04g0125100	Hypothetical protein	2
Os04g0125700	Legume lectin, beta domain containing protein	13
Os04g0126500	Hypothetical protein	6
Os04g0126800	Hypothetical protein	1
Os04g0126900	Conserved hypothetical protein	9
Os04g0127100	Proteinase inhibitor 19, subtilisin propeptide domain	55
Os04g0127300	Peptidase S8 and S53, subtilisin, kexin, sedolisin domain	22
Os04g0127500	EGF-like calcium-binding domain containing protein	3
Os04g0127800	Conserved hypothetical protein	18
Os04g0128300	Hypothetical protein	26
Os04g0128400	Cytochrome P450 family protein	1
Os04g0128700	Conserved hypothetical protein	17
Os04g0128900	Flavin monoxygenase-like protein floozy	40
Os04g0129200	3'-5' exonuclease domain containing protein	3
Os04g0129300	YT521-B-like protein family protein	12
Os04g0129500	Sec23/Sec24 zinc finger domain containing protein	76

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Os04g0129600	Conserved hypothetical protein	6
Os04g0129900	Dynamin family protein	24
Os04g0131900	UDP-glucose:sterol glucosyltransferase	30
Os04g0132300	AAR2 family protein	24
Os04g0132400	Conserved hypothetical protein	1
Os04g0132500	Protein kinase domain containing protein	146
Os04g0136700	CBS domain containing protein	39
Os04g0136900	Conserved hypothetical protein	15
Os04g0137100	Pectate lyase (Fragment)	39
Os04g0137500	Vocuolar ATP synthase subunit	27
Os04g0141400	Legume lectin, beta domain containing protein	1
Os04g0142400	Conserved hypothetical protein	34
Os04g0146800	Protein of unknown function DUF594 family protein	3
Os04g0149400	Hypothetical protein	26
Os04g0150300	Conserved hypothetical protein	35
Os04g0150400	Conserved hypothetical protein	35
Os04g0151600	Hypothetical protein	4
Os04g0151800	Argonaute and Dicer protein, PAZ domain	61
Os04g0151900	Conserved hypothetical protein	33
Os04g0152500	Protein of unknown function DUF594 family protein	4
Os04g0154800	Protein of unknown function DUF594 family protein	5
Os04g0155200	Conserved hypothetical protein	2
Os04g0161100	Conserved hypothetical protein	4
Os04g0162100	Conserved hypothetical protein	64
Os04g0162300	Hypothetical protein	3
Os04g0162400	Hypothetical protein	5
Os04g0162500	Zn-finger, C2H2 type domain containing protein	14
Os04g0162600	Conserved hypothetical protein	24
Os04g0163800	Protein of unknown function DUF594 family protein	14
Os04g0164900	Starch debranching enzyme	92
Os04g0165000	Thaumatin, pathogenesis-related family protein	71
Os04g0165300	Conserved hypothetical protein	15
Os04g0165400	Hrf1 family protein	6
Os04g0165500	Conserved hypothetical protein	15
Os04g0165600	Peptidase S26A, signal peptidase I family protein	6
Os04g0165700	Cysteine synthase	128
Os04g0166000	Conserved hypothetical protein	9
Os04g0166300	Non-protein coding transcript	14
Os04g0166400	AT-rich interaction region domain	31
Os04g0166500	Non-protein coding transcript	3
Os04g0166600	Conserved hypothetical protein	1
Os04g0168000	Conserved hypothetical protein	22
Os04g0168100	Zn-finger, C2H2 type domain containing protein	23
Os04g0168300	Hypothetical protein	19
Os04g0169100	Ethylene receptor	11
Os04g0169300	Conserved hypothetical protein	5
Os04g0169500	Hypothetical protein	4

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Os04g0170500	Zn-finger, CCHC type domain containing protein	4
Os04g0171600	Cytochrome P450 79A1	11
Os04g0172100	Hypothetical protein	7
Os04g0172600	Non-protein coding transcript,putative npRNA	94
Os04g0173300	Hypothetical protein	114
Os04g0173800	Lectin precursor (Agglutinin)	11
Os04g0174100	E-class P450, group I family protein	7
Os04g0174200	Hypothetical protein	47
Os04g0174800	Protein prenyltransferase domain	3
Os04g0174900	Phospholipase/Carboxylesterase family protein	3
Os04g0175000	Hypothetical protein	29
Os04g0175500	Transferase family protein	20
Os04g0175700	Hypothetical protein	59
Os04g0176100	Caffeic acid 3-O-methyltransferase	1
Os04g0176300	Codon recognized: UCA	12
Os04g0177300	SNF2-related domain containing protein	5
Os04g0177400	Riboflavin biosynthesis protein RibD family protein	3
Os04g0177600	Actin/actin-like family protein	61
Os04g0178300	Copalyl diphosphate synthetase	38
Os04g0178400	Cytochrome P450 CYP99A1	10
Os04g0179100	Glucose/ribitol dehydrogenase family protein	10
Os04g0179200	Stem secoisolariciresinol dehydrogenase	19
Os04g0179700	Terpene synthase	7
Os04g0180300	Conserved hypothetical protein	1
Os04g0180400	Cytochrome P450 CYP99A 1 (EC 1.14.-.-) (Fragment)	37
Os04g0180900	Hypothetical protein	21
Os04g0181100	Surfeit locus 4-related family protein	69
Os04g0182200	2OG-Fe(II) oxygenase domain containing protein	26
Os04g0182300	Peptidase S8 and S53,subtilisin,kexin,sedolisin domain	3
Os04g0182600	Conserved hypothetical protein	48
Os04g0182800	Electron transfer flavoprotein beta-subunit (Beta-ETF)	7
Os04g0182900	Amidase family protein	1
Os04g0183100	Conserved hypothetical protein	22
Os04g0183300	Amidase family protein	1
Os04g0183500	Amidase family protein	9
Os04g0184100	Amidase family protein	26
Os04g0185500	Zn-finger, RING domain containing protein	163
Os04g0186400	Phosphate transporter 6	37
Os04g0187800	Conserved hypothetical protein	3
Os04g0188400	Hypothetical protein	8
Os04g0188500	Hypothetical protein	6
Os04g0189400	Gamma Purothionin family protein	7
Os04g0190000	Conserved hypothetical protein	2
Os04g0191000	Retrotransposon gag protein family protein	13
Os04g0191400	Conserved hypothetical protein	36
Os04g0191600	F-box protein interaction domain	18
Os04g0191800	Actin-crosslinking proteins family protein	14

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Os04g0192200	Conserved hypothetical protein	52
Os04g0192800	En/Spm-like transposon proteins family protein	11
Os04g0193300	Cyclin-like F-box domain containing protein	15
Os04g0194000	Cyclin-like F-box domain containing protein	12
Os04g0194400	Transferase family protein	2
Os04g0194500	ABC transporter-like protein	18
Os04g0194600	PCFI	4
Os04g0195000	Vacuolar assembly protein VPS41 homolog	59
Os04g0196200	O-methyltransferase, family 2 domain	9
Os04g0196300	Hypothetical protein	5
Os04g0196600	Protein of unknown function DUF594 family protein	15
Os04g0197200	Protein kinase domain containing protein	9
Os04g0200700	Conserved hypothetical protein	4
Os04g0201200	Peptidase M14, carboxypeptidase A family protein	6
Os04g0201500	Amino acid/polyamine transporter II family protein	6
Os04g0201900	Curculin-like (mannose-binding) lectin domain	4
Os04g0202200	Non-protein coding transcript	23
Os04g0202300	Protein kinase family protein	2
Os04g0202800	Protein kinase domain containing protein	5
Os04g0203100	Protein of unknown function DUF239 domain	4

9. Multiple searching for the contigs not finding any matches.
10. Assigning the contigs as unknown for not finding any matches.

RESULTS AND DISCUSSION

Expressed Sequence Tags has been the most widely sequenced moiety since 1991. ESTs can be used to determine the expression forms in a genome, identify the alternatively spliced forms, to identify new genes. The present study was aimed at extracting information from these moieties and to check which ESTs belong to a certain gene. The organism used for the study was the model plant for genomics and comparative genomics studies, *Oryza sativa*.

A total of 206 genes (Table-1) have been annotated from the EST sequences present on this chromosome 4. A single gene is associated with different ESTs. These genes have various functions and containing different types of proteins which are used in making different types of molecular markers.

The table describes the following terms :

1. Predicted gene : The gene belong to a certain EST. This gene tells about the function and it is predicted from a EST and it is associated with various ESTs. This gene ID is present in the GenBank.This gene help us to predict molecular markers from it.

2. Annotation info : The function of the predicted gene whether this gene is a protein or a domain. This information is helpful in various types of Research in bioinformatics and biotechnology.

3. No. of ESTs : How many ESTs are associated to the predicted gene. This tells about that various no. of ESTs are associated with the above predicted gene.

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D^2 Analysis in Some Varieties of Tomato

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Genetic divergence among six varieties of tomato growing in and around Jharkhand was studied with the help of karyotype analysis. Genetic distances were measured following D^2 – statistics and grouped into three distinct constellations. The inter and intra constellation distance represent the index of genetic diversity among the constellations. The constellation II showed the highest divergence from constellation III (4.283) suggesting thereby that the genotypes belonging to constellation II and III may be undertaken in a hybridization programme for getting good segregants.

Keywords : Genetic divergence, Karyotype, Inter and Intra constellation.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most important vegetable crop grown throughout the world for its edible fruit. The genus *Lycopersicon esculentum* Mill belongs to family Solanaceae. It is one the most popular and widely grown vegetable ranking second in importance to potato in many countries. In India, tomatoes can be grown nearly throughout the year since favourable climate conditions are available in one part or another. Tomatoes are packed with vitamin C, A, Potassium and fiber. Besides its economic value, tomatoes have great medicinal properties. Research findings suggest that lycopene, a caroteniod present in tomato is the natural cancer fighting agent.

Knowledge of genetic diversity among plant populations and its quantitative assessment usually help a breeder in choosing desirable parents for breeding programme. The present study was undertaken to assess the extent of genetic diversity in six varieties of tomato. The D^2 – statistics (Mahalanobis, 1936, 1949) had been applied to find out the genotypic closeness among different varieties of tomato.

MATERIAL AND METHODS

Seeds of different varieties of tomato were collected from local nurseries. They were cleaned and kept in separate labeled petridishes for germination under aseptic condition. Young root apices of 2 - 5 mm were preserved after going through a series of treatments. Slides were prepared using acetocarmine squash technique. Ten well separated mitotic metaphase chromosome plates were studies and data were recorded from individual chromosome in each variety. The data were analyzed statistically using Mahalanobis (1936, 1949) formula :

1. **Absolute Length :** This was the summation of short arm and long arm of the chromosomes.

2. **Relative Length :** The relative length of each chromosome was calculated by dividing the length of chromosome pair with length of longest chromosome pair and multiplying it by 100.

$$\text{Relative length} = \frac{\text{Length of chromosome pair}}{\text{Length of longest chromosome pair}} \times 100$$

3. **Angular Value :** The angular value of the chromosome was calculated by conversion of relative length in per cent into degree according to the table by C.I. Bliss as reproduced in the statistical methods by Snedecor (1959).

4. **Log Value :** The ratio of long arm and short arm of the chromosomes were converted into logarithmic scale.

D^2 Analysis : Length of individual chromosome was transferred into angular scale (p) and Z. The ratio of long arm and short arm was transferred into logarithmic scale (q). The chromosomes were regarded as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 according to their values of absolute length (Table 1). Treating 'p' as abscissa and 'q' as ordinate, each chromosome could plotted as point 'p' and 'q'. The graph comes to be a straight line p/q representing line gradient of the line (= tanØ) length of the line = $(p^2 - q^2)^{1/2} = Z$. The ratio tan θ/Z = K gives a good key to all the quantitative characters of an individual chromosome. Let K_1^x and K_1^y be the K values of the variety 'X' and 'Y' respectively than for 12 independently variable chromosomes, the degree of divergence between the variety 'X' and 'Y' will be equal to $D^2 = \Sigma_{12}^{1} (K_1^x - K_1^y)^2$.

RESULTS AND DISCUSSION

Karyotype analysis revealed uniformity in the diploid chromosome complemnet *i.e.,* 2x = 24 in all the six varieties of tomato under investigation (Table 1). The chromosomes were of very small size but variation existed in shape and size of individual chromosomes. The highest chromosome length

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Table 1 : The relative length (in μ) of individual chromosomes and its conversion in to angular value (a) and the ratio of long arm/short arm and its conversion into logarithmic scale (b).

Varieties of *Lycopersicon esculentum* Mill		CHROM I	CHROM II	CHROM III	CHROM IV	CHROM V	CHROM VI	CHROM VII	CHROM VIII	CHROM IX	CHROM X	CHROM XI	CHROM XII
L. esculentum Mill Var. Pusa Early Dwarf	a.	13.64 ± 0.16 (21.64)	11.25 ± 0.16 (19.64)	10.08 ± 0.19 (18.53)	9.15 ± 0.13 (17.66)	8.92 ± 0.14 (17.36)	8.59 ± 0.11 (17.05)	7.99 ± 0.09 (16.43)	7.66 ± 0.08 (16.11)	6.92 ± 0.13 (15.23)	6.26 ± 0.11 (14.54)	5.16 ± 0.06 (13.18)	4.40 ± 0.04 (12.11)
	b.	2.41 ± 0.14 (0.38)	2.56 ± 0.21 (0.40)	1.92 ± 0.12 (0.28)	2.10 ± 0.17 (0.32)	1.89 ± 0.13 (0.27)	1.83 ± 0.10 (0.26)	2.13 ± 0.11 (0.32)	1.98 ± 0.05 (0.29)	2.41 ± 0.16 (0.38)	2.53 ± 0.21 (0.40)	2.51 ± 0.17 (0.39)	3.15 ± 0.04 (0.54)
L. esculentum Mill Var. Local Variety	a.	14.50 ± 0.13 (22.38)	12.46 ± 0.13 (20.70)	10.74 ± 0.15 (19.09)	9.78 ± 0.08 (18.24)	9.13 ± 0.13 (17.56)	8.52 ± 0.08 (16.95)	7.80 ± 0.07 (16.22)	7.34 ± 0.12 (15.68)	5.73 ± 0.09 (13.81)	5.37 ± 0.09 (13.44)	4.91 ± 0.06 (12.79)	4.05 ± 0.07 (11.68)
	b.	2.55 ± 0.14 (0.40)	2.28 ± 0.17 (0.35)	2.10 ± 0.20 (0.32)	2.41 ± 0.41 (0.38)	1.99 ± 0.002 (0.29)	2.18 ± 0.09 (0.33)	2.23 ± 0.11 (0.34)	2.34 ± 0.14 (0.36)	2.93 ± 0.27 (0.46)	2.70 ± 0.20 (0.43)	2.82 ± 0.23 (0.45)	2.43 ± 0.17 (0.38)
L. esculentum Mill Var. Selection 22	a.	13.35 ± 0.10 (21.39)	11.31 ± 0.03 (19.61)	10.19 ± 0.09 (18.53)	9.33 ± 0.04 (17.76)	8.61 ± 0.06 (17.05)	8.50 ± 0.06 (16.95)	7.79 ± 0.09 (16.11)	7.54 ± 0.09 (15.80)	6.82 ± 0.11 (15.12)	6.28 ± 0.10 (14.42)	5.45 ± 0.06 (13.44)	4.77 ± 0.07 (12.52)
	b.	2.39 ± 0.08 (0.37)	2.07 ± 0.05 (0.31)	2.29 ± 0.11 (0.35)	2.08 ± 0.11 (0.31)	2.31 ± 0.09 (0.36)	2.18 ± 0.10 (0.33)	2.23 ± 0.09 (0.34)	2.01 ± 0.08 (0.30)	2.45 ± 0.23 (0.38)	2.80 ± 0.24 (0.44)	2.66 ± 0.16 (0.42)	2.74 ± 0.14 (0.43)
L.esculentum Mill Var. Pusa Ruby	a.	14.90 ± 0.15 (22.71)	12.25 ± 0.70 (20.53)	11.02 ± 0.14 (19.37)	9.11 ± 0.15 (17.56)	8.17 ± 0.06 (16.64)	7.96 ± 0.07 (16.43)	7.88 ± 0.10 (16.32)	6.53 ± 0.13 (14.77)	6.12 ± 0.10 (14.36)	5.72 ± 0.004 (13.81)	5.68 ± 0.01 (13.81)	4.61 ± 0.07 (12.39)
	b.	4.30 ± 0.98 (0.63)	2.43 ± 0.20 (0.38)	2.71 ± 0.56 (0.43)	2.62 ± 0.40 (0.41)	2.57 ± 0.36 (0.40)	2.86 ± 0.40 (0.45)	2.52 ± 0.28 (0.40)	2.75 ± 0.19 (0.43)	2.90 ± 0.15 (0.46)	3.04 ± 0.04 (0.48)	3.11 ± 0.01 (0.49)	2.70 ± 0.13 (0.43)
L. esculentum Mill Var. Punjab Kesari	a.	14.04 ± 0.05 (21.97)	12.57 ± 0.05 (20.70)	10.52 ± 0.06 (18.91)	9.62 ± 0.06 (18.05)	8.59 ± 0.03 (16.95)	8.31 ± 0.05 (16.74)	7.86 ± 0.06 (16.22)	7.04 ± 0.10 (15.34)	6.06 ± 0.03 (14.18)	5.77 ± 0.10 (13.81)	5.28 ± 0.06 (13.18)	4.29 ± 0.07 (11.83)
	b.	2.35 ± 0.08 (0.37)	2.08 ± 0.05 (0.31)	2.26 ± 0.12 (0.35)	2.39 ± 0.16 (0.37)	2.13 ± 0.09 (0.32)	2.13 ± 0.09 (0.32)	2.53 ± 0.15 (0.40)	2.73 ± 0.18 (0.43)	3.21 ± 0.10 (0.50)	2.92 ± 0.07 (0.46)	2.78 ± 0.09 (0.44)	2.48 ± 0.12 (0.39)
L.esculentum Mill Var. Selection 18	a.	13.38 ± 0.07 (21.39)	10.27 ± 0.05 (18.63)	10.27 ± 0.05 (18.53)	10.13 ± 0.02 (18.53)	10.13 ± 0.02 (18.53)	7.50 ± 0.11 (15.80)	6.93 ± 0.05 (15.23)	6.78 ± 0.02 (14.89)	6.78 ± 0.02 (14.89)	6.54 ± 0.02 (14.77)	6.54 ± 0.02 (14.77)	4.79 ± 0.005 (12.52)
	b.	3.50 ± 0.42 (0.54)	2.07 ± 0.07 (0.31)	2.07 ± 0.07 (0.31)	2.18 ± 0.18 (0.33)	1.94 ± 0.05 (0.28)	2.80 ± 0.14 (0.44)	3.14 ± 0.14 (0.49)	2.85 ± 0.14 (0.45)	2.85 ± 0.14 (0.45)	2.93 ± 0.07 (0.46)	2.93 ± 0.07 (0.46)	2.45 ± 0.04 (0.38)

a = Relative Length ± (S.E.) = Angular transformation from % to degrees, b = Long arm/short arm ± (S.E.) = Logarithmic scale (Long arm/short arm)
(The chromosome numbers have been named from 1 - 12 in accordance with their length in different varieties).

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Table 2 : K - value of individual chromosomes of six varieties of *Lycopersicon esculentum* Mill.

Sl.	Varieties under consideration	CHROM I	CHROM II	CHROM III	CHROM IV	CHROM V	CHROM VI	CHROM VII	CHROM VIII	CHROM IX	CHROM X	CHROM XI	CHROM XII
1.	*Lycopersicon esculentum* Mill Var. Pusa Early Dwarf V_1	2.62	2.45	3.53	3.10	3.61	3.81	3.04	3.37	2.61	2.48	2.50	1.82
2.	*Lycopersicon esculentum* Mill Var. Local Variety V_2	2.46	2.79	3.10	2.61	3.35	2.95	2.87	2.70	2.14	2.30	2.21	2.58
3.	*Lycopersicon esculentum* Mill Var. Selection 22 V_3	2.64	3.16	2.77	3.14	2.74	2.95	2.87	3.29	2.56	2.28	2.34	2.27
4.	*Lycopersicon esculentum* Mill Var. Pusa Ruby V_4	1.58	2.59	2.31	2.39	2.44	2.19	2.49	2.27	2.16	2.06	2.02	2.31
5.	*Lycopersicon esculentum* Mill Var. Punjab Kesari V_5	2.69	3.14	2.82	2.64	3.04	3.04	2.44	2.28	1.96	2.14	2.24	2.52
6.	*Lycopersicon esculentum* Mill Var. Selection 18 V_6	1.83	3.16	3.16	2.95	2.95	2.23	2.00	2.19	2.19	4.83	4.83	2.56

Table 3 : Generalised distance and clustering pattern of six varieties of *Lycopersicon esculentum* Mill.

Sl. No.	V_1	V_2	V_3	V_4	V_5	V_6
1.	V_2 - 2.768	V_5 - 1.258	V_5 - 2.038	V_5 - 3.144	V_2 - 1.258	V_2 - 15.617
2.	V_3 - 2.886	V_1 - 2.768	V_1 - 2.886	V_2 - 3.389	V_3 - 2.038	V_5 - 15.816
3.	V_5 - 4.758	V_4 - 3.389	V_2 - 3.921	V_3 - 4.403	V_4 - 3.144	V_3 - 16.297
4.	V_4 - 9.459	V_3 - 3.921	V_4 - 4.403	V_1 - 9.459	V_1 - 4.758	V_4 - 18.324
5.	V_6 - 18.369	V_6 - 15.617	V_6 - 16.297	V_6 - 18.324	V_6 - 15.816	V_1 - 18.369

(In the Table Serial No. 1 is the closest and chronologically Serial No. 5 is farthest).

(4.10 μ) was recorded in the variety Pusa Early Dwarf and was lowest (0.98μ) in the variety Selection 18. The rest were between these two values. The maximum total chromatin (30.05 μ) length was observed in the variety Pusa Early Dwarf, while it was minimum (20.92μ) in the variety Selection 18. The rest were between these two values. While comparing the total chromatin length, the species or varieties having greater chromatin length were considered to be primitive and lesser chromatin length was treated as advanced (Stebbins, 1971). Thus in present investigation variety Pusa Early Dwarf was considered most primitive with greater total chromatin length and variety Selection 18 was considered most advanced with lesser total chromatin length. Karyotype analysis indicates that nearly sub median type of chromosomes were common in all the varieties except variety Pusa Ruby where 2 chromosomes were sub median along with 22 nearly sub median type of chromosomes. According to Stebbins (1951) the species or varieties having greater number of sub median chromosomes should be treated as more evolved then those having lesser number of sub median chromosome. Therefore, among the six varieties, Pusa Ruby should be treated more evolved than others.

The analysis of genetic divergence in different varieties of *Lycopersion esculentum* Mill under investigation has been calculated by taking the value of long arm and short arm and also be using K value (Table 3) of individual chromosome. All the varieties were grouped into three constellations (Figure 1). The constellation C I consists of three varieties of *Lycopersicon esculentum* Mill V_2, V_3 and V_5 which represents Local Variety, Selection 22, and Punjab Kesari. The constellation C II consists of two varieties V_1 and V_4 which represents the variety Pusa Early Dwarf and Pusa Ruby. The constellation C III consists of one variety V_6 which represents the variety Selection 18. The analysis made in view of genetic divergence indicated that intra constellar distance between the constellations C I, C II and C III were 4.554, 3.076 and 0.000 (Figure 1). The inter constellation distance between C I - C II, C II - C III and C I - C III were 1.886, 4.283 and 3.99 respectively (Figure 1). The highest and lowest D^2 values in the inter constellar divergene were observed between C II to C III and C I to C III respectively. The intra and inter constellar distance represent the index of genetic diversity among the constellations. The genotypes grouped together are less divergent than one grouped into different clusters. Therefore, crossing between distant clusters might be more useful. The constellation C I recorded a maximum intra constellation distane 4.554, where as, cluster C III had zero intra constellation distane. With respect to inter cluster distance constellation showed the highest divergence from constellation III (4.283) suggesting thereby that the genotype belonging to constellation II and III may be undertaken in a hybridization programme for getting good segregants Earlier Sachan and Sharma (1971) and Sharma and Verma (2001) have reported the multivariate analysis of genetic divergence in tomato. The D^2 statistics are better exploited in breeding programmes. The highest inter constellar distane suggested the genetic recombination between the genotypes of two clusters and will result in hetrosis

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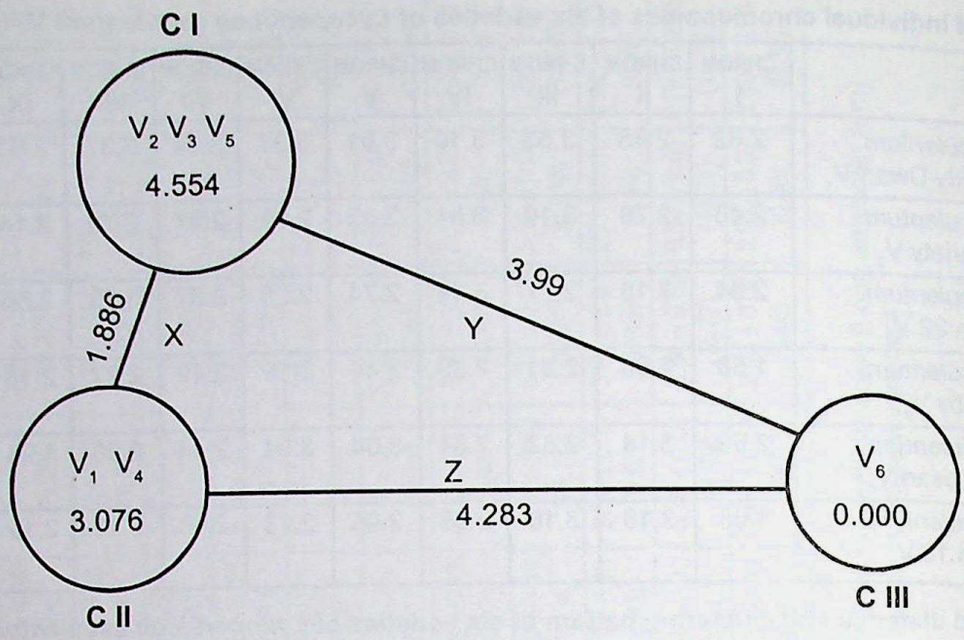

Figure 1 : Generalized distance between and within constellations on the basis of D²-value among six varieties of *Lycopersicon esculentum* Mill.

as supported by Mahalanobis (1936, 1949); Rao (1952); Renthlei *et al.* (1994); Kumar and Akhoury (1996); Singh, Jagtar *et al.* (2005); and Kumar *et al.* (2006).

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Pharmacognostical Studies on *Solanum nigrum* L.

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The present study is the first Pharmacognostical investigations on *Solanum nigrum* L. (Solanaceae). Anatomical, histochemical, phytochemical, fluorescence and ash analysis were carried out in leaves, roots and fruits. The values calculated/ data collected could be used for the identification of powdered drug of this taxon.

Keywords : *Solanum nigrum* L.- Pharmacognostical studies.

INTRODUCTION

Solanum nigrum L. syn. *Solanum rubrum* Roxb. known as Black night- shade (English), Makoy (Hindi), Kakamaci (Sanskrit) and Manathakkali (Tamil) is a common plant in India and Ceylon. It is an unarmed shrub of approximately 1m long, branches zigzag, leaves broadly ovate-elliptic, inflorescences umbel, extra - axillary, calyx cupular, corolla white, berries black and seeds discoid. Sharma *et al.* (1995) carried out the phytochemical analysis in the leaves of this plant and extracted few steroid glycosides. The leaves are rich in protein (22%) and contain free amino acids (6.85%), riboflavin, nicotinic acid, vitamin C, β-carotene (0.74 mg/100mg), traces of ∞-carotene and critic acid (Anonymous, 2003). Mallika and Shyamala (2004) substantiated the antiulcerogenic effect of the methanolic extract of berries of *Solanum nigrum*. Juneja *et al.* (2005) showed that solasonine and solamargine present in the leaves, stem and young fruits of this plant have the anti-inflammatory property. Dhellot *et al.* (2006) in their physico-chemical study estimated the amount of lipids in the dry matter content of the seeds. Lanzhou *et al.* (2007) extracted two pregnane saponins, solanigroside A and solanigroside B from 60% ethanolic extract of the dried plant. Akubugwo *et al.* (2007) in their phytochemical work estimated the nutritional potential of the leaves and seeds. The results indicated the protein content of the leaves and the seeds as 24.90% and 17.63% respectively. As not much work has been carried out on this plant's anatomy, histochemistry and phytochemistry, the present investigation was taken up.

MATERIAL AND METHODS

Anatomical works were carried out in fresh specimen. Mature leaves were cleared by using 5% NaOH and chloral hydrate solution, washed in water, stained and mounted in 50% glycerine. Histochemical colour reactions were observed by treating free-hand sections with different reagents. Phytochemical tests were done with dried powder drugs. Fluorescence analysis was done using various solvent extracts of the powder drug in day light and UV light-365 nm (Chase and Pratt, 1949). Phenolic content was determined by Folin-Ciocalteu Phenol assay method (Singleton *et al.*, 1999). For the determination of flavonoid content, procedure outlined by Zhishen *et al.* (1999) was followed. Total ash, acid insoluble ash and water soluble ash values were calculated following Khandelwal *et al.* (1996) taking 2 g of crude leaf powder.

RESULTS AND DISCUSSION

A combination of characters such as epidermal cell size, stomatal size, their distribution, stomatal index, presence or absence of hairs, their types, palisade ratio, venation pattern, vein-islet numbers, veinlet termination number are found to be very significant micro-morphological characters in the identification of crude drugs. Histochemical and phytochemical studies carried out in a plant are found to be very significant in pharamacognostic identification for crude drugs. Fluorescence

Table 1 : Quantitative measurements of foliar epidermal structures.

S.N.	QUANTITATIVE VALUES		ABAXIAL	ADAXIAL
1.	Epidermal cell size (μm)	(L)	57.75	78.54
		(B)	19.80	24.09
2.	Epidermal cell/ mm²		1209.78	812.16
3.	Stomatal size (μm)	(L)	23.76	21.78
		(B)	17.49	15.51
4.	Stomata/ mm²		269.3	102.93
5.	Stomatal index		18.26	11.64
6.	Palisade ratio		-	4.8
7.	Vein-islet number / mm²		135.36	-
8.	Vein termination number / mm²		63.45	-
9.	Glandular hair size (μm)	(L)	57.75	-
		(B)	21.75	-
10.	Non-glandular hair size (μm)	(L)	192.06	-
		(B)	21.45	-

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Table 2 : Histochemical analysis.

S.N.	TEST FOR	CHEMICALS / REAGENTS USED	LEAF	ROOT	FRUIT
1.	Lignin	Sections mounted in 1% neutral aqueous potassium permanganate, washed in water, placed in 2% HCl for 2 mts, washed in water, mounted in dilute ammonia solution	++	++	++
2.	Tannins	Sections mounted in 1% solution of ferric chloride	-	+	-
3.	Mucilage	Sections mounted in methylene blue	++	++	++
4.	Starch	Sections mounted in 1% iodine solution	++	++	-
5.	Alkaloid	Sections mounted in Meyer's reagent	-	-	-
6.	Lipids and Lipoproteins	Sections mounted in 70% ethanol for 1-2 mts, stained in Sudan black B at 60°C for 1 hr, rinsed in ethanol and washed in water	++	++	+
7.	Proteins	Sections stained in aqueous solution of picric acid for 24 hrs.	++	-	++

++: Markedly present, +: Moderately present, -: Absent

Table 3 : Phytochemical analysis.

S.N.	TEST FOR	CHEMICALS / REAGENTS USED	LEAF	ROOT	FRUIT
1.	Flavonoids	To the substance few drops of alcohol, 10% sodium hydroxide or ammonia added	++	+	-
2.	Glycosides	Substance mixed with little anthrone, few drops of con. sulphuric acid added, made as paste, warmed gently over water bath	++	++	++
3.	Phenol	To the substance few drops of alcohol and ferric chloride solution added	++	-	-
4.	Saponins	Substance vigorously shaken with water	++	++	++
5.	Sugars	To the substance Fehling's solution A and B added	-	-	-

++: Markedly present, +: Moderately present, -: Absent

Table 4 : Fluorescence analysis.

S.N.	Treatment	Leaf		Root		Fruit	
		Day light	uv 365nm	Day light	uv 365nm	Day light	uv 365nm
1.	Powder as such	Dark green	Black	Light yellow	Dark yellow	Brown	Brownish black
2.	Powder + Hexane	Dark yellow	Orange	Yellow	Pink	Yellow	Pink
3.	Powder + Benzene	Marsh green	Pinkish red	*	*	*	Pale Yellow
4.	Powder + Chloroform	Dark green	Dark red	Pale yellow	*	Pale yellow	Yellow
5.	Powder + Acetone	Light green	Pinkish Orange	*	*	*	*
6.	Powder + Alcohol	Light green	Orange	*	*	*	*
7.	Powder + Water	Brown	Violet	*	*	Dark Brown	Pinkish green
8.	Powder + IN HCl	Light Brown	Violet	Pale yellow	*	Brownish yellow	Dark brown
9.	Powder + 50% H_2SO_4	Dark green	Black	Pale brown	Black	Dark brown	Black
10.	Powder + 10% Alcoholic NaOH	Dark green	Pink	Reddish orange	Reddish brown	Brown	Fluorescence yellow
11.	Powder + 10% Aqueous NaOH	Brown	Pinkish red	Dark yellow	Fluorescence yellow	Brown	Dark Brown

*No Characteristic colour

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Table 5 : Phenolic and Flavonoid content in leaves.

S.N.	Reagent used	Phenolic content	Flavonoid content
1.	max (nm) methanol	257.5	360.0
2.	Methanol + Sodium acetate	270.0	410.5
3.	Methanol + Sodium acetate + O.Boric acid	263.5	394.0
4.	Methanol + Aluminium Chloride	272.5	305, 428.5
5.	Methanol + Aluminium Chloride + HCl	267.0	362.5, 395.0

Ash value of leaves:
Total ash: 13.4% w/w
Acid insoluble ash: 1.8% w/w
Water soluble ash: 11.6% w/w

analysis utilizes the fluorescence produced by a compound in ultraviolet light for analytical evaluation. Speaking generally, fixed oils and fats fluorescence leastly, waxes more strongly and mineral oils most of all. The ash value furnishes a basis for judging the identify and cleanliness of a drug in a powder form. The anatomical, histochemical and phytochemical values including ash value and the value of phenolic and flavonoid content furnished above are the standard values for the leaf, root and fruit drugs of *Solanum nigrum* L. and they could be successfully used for the identification of powdered drug of this taxon.

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Ethnobotanical Observation on Wild Tuberous Plants of Satara District (Maharashtra)

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The present paper gives an account of 29 tuberous plants of ethnobotanical interest of satara district. Rural people use the tuberous plants for various purposes in their daily life. Health, vitality and longevity enjoyed by the people have been attributed by them to these wild tuberous plants. Therefore identification and utility of these tuberous plants and compilation of a database on local information held by the people is stressed. Ethnobotanical information about tuberous plants is given by mentioning their botanical name, family, local name, flowering and fruiting, place of occurrence and ethnomedicinal uses. Tribal group met with are ramoshis, kaikaris, mange and mhars.

Keywords : Tuberous plants, Ethnobotany, Food plants, Medicinal plants, Satara.

INTRODUCTION

Tuberous plants characteristically have a storage organ on or below the soil surface. This organ may be a true bulb, corm, tuber, tuberous root, rhizome, stolon or pseudobulb. Carbohydrates and nutrient reserve are stored in these organs. Ethnobotanical survey of Satara district revealed that the rural communities use a number of tuberous plants, which are commonly available in and around their habitat. The rural people and tribal communities such as ramoshis, kaikaris, mange, and mhars use various tuberous plants for dietary and therapeutic purposes in their daily life.

Satara is one of the 31 districts of Maharashtra and part of Western Maharashtra. The Sahyadri range or main range of Western Ghats runs North and South along the western edge of Satara district which located at 17°,5 to 18°, 11 N and 73°, 33 to 74°, 54 E. It occupies area about 10484 sq.Km. It is the hilly district and Annual rainfall is 1426 mm which different in various places, among which Mahableshwar receives highest rainfall while Man taluka gets minimum rainfall. The temperature of the district is maximum 37.5°C and minimum 11.6°C. The soil in satara district is red being of lateratic origin. In Satara district many places are rich with variety of flora such as Mahableshwar, Pachagni, Koynanagar, Kas, Kartikswami ghats, Thoseghar, Wai. In field of ethnobotany some workers significantly contributed in Indian medicinal plants from this region (Vartak 1959, Ved Prakash & Mehrotra 1987, Upadhye *et al.*, 1994, Mahekar and Yadav 2006).

Since, studies on ethno-food and ethno-medicinal plants has been carried out all over the world. A perusal of the literature reveals that there is still an ethnobotanical gap in knowledge about tuberous plants in these regions. The present paper therefore is an attempt to fill this gap. Apart from it and some new ethnobotanical uses have also been reported.

METHODOLOGY

Several field trips were undertaken throughout the district at different seasons and collect more information. The information was accured after discussion with several persons, village head, elder women and other local informants because they are the only source to collect the information about local plant name and their use. Plant specimens were collected and identified using floras. The information about the food and medicine plants is given by mentioning their botanical name, family, local name, flowering and fruiting, place of occurrence and ethnobotanical uses.

ENUMERATION

The plant species are arranged alphabetically by their binomial along with family followed by local name, place of occurrence, flowering, fruiting and ethnobotanical uses.

Amorphophallus paeoniifolius (Dennst.) Nicols., Araceae, L.N. Jangali suran.
Fl. & Fr. June-Sept., Common in shady places or open places.
Uses: Corm used externally 7-8 days in rheumatic swelling. Whole plant used in enlargement of spleen, blood diseases, elephantiasis & leucoderma.

Asparagus racemosus Willd., Liliaceae, L.N. Shatavari, Ashwal.
Fl. & Fr. June-Nov., Common on barren and rocky soil under shade of trees.

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Uses: 1-Cup Root juice given 7-8 days to the women's suffering from leucorrhoea & used in debility, night blindness, throat complaints, epilepsy.

Brachystelma edulis Coll., Asclepiadaceae, L.N. Hanuman batata, Galya.

Fl. & Fr. July-Sept., on partially degraded hill slopes and open hill tops.

Uses: Tubers used for bodily discomfort. Used for headache, stomach ache, and colds in children. Eaten as food supplements.

Bryonia dioica Jacq. Cucurbitaceae., L.N. Chambhar gonda.

Fl. & Fr. May-Aug., Rare in semievergreen forest.

Uses: Roots given in stomachache. Chewed tuber caused abortion to pregnant women.

Ceropegia bulbosa Roxb.,Asclepiadaceae., L.N. Haman, Khandwel.

Fl. & Fr. Aug-Nov., Frequent admist bushes on hill slopes.

Uses: Tubers used as tonic & digestive. Boiled tuber pulped with milk form a sweet mucilaginous mixture which should be highly nutritious. 1-Cup tuber juice taken 7-8 days given in leucorrhoea, seminal debility and bowel complaints of children. Tuber eaten as raw or vegetable.

Ceropegia hirsuta Wt. & Arn., Asclepiadaceae. L.N. Kharpudi, Khartundi.

Fl. & Fr. July-Nov., Frequent on hill slopes.

Uses: Whole plant is used as an analgesic. Fresh root given every day for 4-5 days for treating stomach disorders. Boiled tubers edible.

Corallocarpus epigaeus (Rottl) Hk.f., Cucurbitaceae., L.N. Mirchikand, Karmuglia, Karvina.

Fl. & Fr. June-Aug., Frequently in bushes on hill slopes.

Uses: Roots applied externally in rheumatism. 1-2 spoon root juice for 3-4 days to cures inflammation and dysentery.

Costus speciosus (Koen) Sm., Zingiberaceae., L.N. Kosht, piway, ratanjyot.

Fl. & Fr. Aug-Feb., Common in moist deciduous and semievergreen forest.

Uses: Rhizome given as a tonic and anthelmintic. Rhizome paste applied externally on skin to remove excess pigmentation on skin after pregnancy.

Cryptocoryne spiralis (Retz.) Fisch., Araceae.,

Fl. & Fr. Aug-Dec., Frequently partially submerged in pools or in margin of ponds & rivers.

Uses: Given infantile vomiting & cough and in case of adults for abdominal complaints & fever.

Cyperus rotundus L., Poaceae., L.N. Nagarmotha.

Fls. & Frts. Sep-Dec., Common weed of wet agriculture fields.

Uses: Applied externally to stop hair loss & in graying of hairs & externally to the breast as a galactogogue. Tuber used as appetizer, stomachic, anthelmintic, useful in leprosy, blood diseases.

Dioscorea bulbifera L., Dioscoreaceae., L.N. Dukkar kand.

Fl. & Fr. Sept.-Jan., Common in bushes in hill forests.

Uses: Tuber used as vegetable. Bulbils given orally in acidity; improve apetite. Useful in dyspensia, urinary discharges, leucoderma, bronchitis, tumours.

Dioscorea oppositifolia L., Dioscoreaceae., L.N. Paspoli.

Fl. & Fr. Aug-Oct., Common in hill forests.

Uses: Root made into paste, heated & applied to reduce swelling. Externally applied in itching.

Drimia polyantha. (Blatt. & McC) Ans. & Ragh., Liliaceae., L.N. Panchankanda.

Fl. & Fr. Mar-Apr., Common in grasses.

Uses: Bulb used as diuretic, bronchitis, dropsy, rheumatism, diseases of nose, internal pains & scabies.

Eulophia nuda Lindl., Orchidaceae., L.N. Amarkand.

Fl. & Fr. Apr-Sept., Common in moist deciduous forests in shady places.

Uses: Tuber given in gas troubles & in acidity, internally in backaches & also used as food. The tuber used as appetizer, useful to tuberculous glands in neck, tumour & in bronchitis.

Flemingia tuberosa Dalz., Fabaceae.

Fl. & Fr. Sept.-Nov., Apparently endemic on plateaus.

Uses: Tubers astringent, useful in dysentery & vaginal discharges.

Gloriosa superba L.,Liliaceae., L.N. Kallavi, Bachang.

Fl. & Fr. Sept.-Nov., Common in hill forests admist bushes.

Uses: Entire tuber tied around the back during labour. Tuber used as anthemintic, abortifacient, also used in ulcer, leprosy.

Hitchenia caulina (Grah.) Baker., Zingiberaceae., L.N. Chava, Chowar.

Fl. & Fr. July-Oct., Common or abundant, in crevices of rocks on plateaus of higher altitudes.

Uses: Tubers given in general debility with milk.

Iphigenia indica (L.) Kunth., Liliaceae., L.N. Markali.

Fl. & Fr. June-Sept., Common on grasslands.

Uses: Tubers externally applied in skin diseases such as itch, elephantiasis, diluted juice with limewater given in colic or abdominal pain.

Ipomoea mauritiana Jacq. Convolvulaceae., L.N. Bhuikolha.

Fl. & Fr. July-Oct., Occasional in moist deciduous forests.

Uses: Orally given in rheumatism. Tubers given with root of *A. racemosus* to increase secreation of milk. Root used as tonic, aphrodisiac, galactogogue, diuretic, stimulant useful in burning sensation, vomiting diseases of the blood, improve voice & complexion.

Leea macrophylla Roxb., Leeaceae., L.N.-Dinda.

Fl. & Fr. July-Oct., Occasional in hill forests.

Uses: Tubers used as vegetable. Roots pasted & applied on wounds & ulcers and also used in itching & elephantiasis.

Momordica cymbalaria Hook., Cucurbitaceae., L.N. Kadvachi.

Fl. & Fr. June-Oct., Occasional in dry region.

Uses: Tubers used to procure abortion.

Momordica dioica Roxb ex. Willd., Cucurbitaceae., L.N. Kartoli.

Fl. & Fr. July-Oct., Frequently in grasses and also on slopes of ghats.

Uses: Root powder given orally in diabetes. Roots given in urinary calculi & also as diuretic. Roots used to stop bleeding from piles. Roots used in heat troubles, urinary calculi as an errhenine in jaundice, cures elephantiasis.

Nymphaea pubescence Willd., Nymphaeaceae., L.N. Kamal.

Fl. & Fr. Throughout the year., Frequent in lakes & ponds.

Uses: Tubers and peduncle eaten as vegetable. Powered roots used for piles as a demulcent, also for dysentery, dypensia & cardiac tonic.

Operculina turpethum (L.)., Convolvulaceae., L.N. Dudhkalami, Nishothar.

Fl. & Fr. Sept-Apr., Common on bushes & in hedges.

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Uses: Tuberous root of white variety used as purgative. Black variety powerful in irritant & drastic putative. Root anathematic useful in ascites, itch, leucoderma, constipation, abdominal troubles, inflammation, fever, anemia, piles.

Pueraria tuberosa (Willd.) DC., Fabaceae., L.N. Bhuikolha, Vidarikand.

Fl. & Fr. Feb-Apr., Frequently in deciduous forests.

Uses: Used to cure rheumatic pains. Tubers given orally in epilepsy. Tuber used as aphrodisiac, tonic, glactogogue, diuretic, clears the voice and cures blood diseases.

Remusatia vivipara (Roxb.) Schott., Araceae., L.N. Rukhal kand.

Fl. & Fr. Apr-Sept., Common in the cleft of trees in ghats & in jungles.

Uses: Tuber given for external application in piles with the mix of *D. bulbifera* bulbils & bark of *Pittosporum dacycaulon.* Root made into ointment with turmeric & used as a remedy for itch. & the juice with cow's urine is considered to be alexipharmic.

Solena amplexicaulis (Lam.) Gandhi., Cucurbitaceae, L.N. Gomati, Gometta.

Fl. & Fr. July-Oct., Frequent in moist deciduous & semievergreen forests.

Uses: Roots given as nutritive food, juice of root used with milk & given in menorrhea. Roots orally given in rheumatism.

Souromatum venosum (Ait) Schott. Araceae., L.N. Loth, Nurki.

Fl. & Fr. March-May., Very Common in shady places along roadside.

Uses: Tubers externally applied in piles with the castor oil. The tubers used as stimulating poultice.

Teramnus labialis (L.F.) Spreng., Fabaceae., L.N. Ranudid.

Fl. & Fr. Sept-Dec., Common in hedges around fields & under the shade of tree.

Uses: Tuberous Root given in rheumatism. Roots given as a tonic & galactogogue. Fruits used as antipyretic, tonic, inflammation, bronchitis, burning sensation, useful in paralysis.

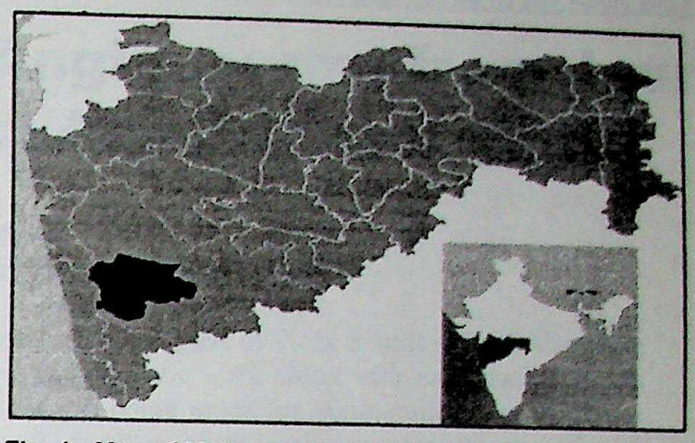

Fig. 1 : Map of Maharashtra showing the study area

DISCUSSION

Presently, rural people are using these wild tuberous plants for their primary health care. Others do not know about these plants. The information regarding medicinal & food uses of plants reported are scrutinized with published literature. However, the medicinal properties & active principles of these plants should be standardized significantly & tested clinically for safe use.

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Medicinal Flora of Angiosperms from Jeur (Bayajabai), District Ahmednagar (M.S.)

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Jeur (Ba) village is rich in deciduous plants of Angiosperms. It is adopted by N. S. S. unit of Radhabai Kale Mahila Mahavidyalaya, Ahmednagar for floristic survey. No floristic work along with medicinal uses has been undertaken so far in this area. Therefore, an attempt is being made for enumerating the important plants. The present work reports 50 species belonging to 27 families. Their local uses were overviewed with the help of local informants and from literature available.

Keywords : Jeur (Ba), Medicinal uses, Flora of Angiosperms.

INTRODUCTION

The village Jeur (Ba) belongs to Ahmednagar district. It is the place where the river Seena rises. Topographically the area is divided into plains and hills. The hilly regions are a treasure of vegetation with diversity of life. The vegetation from catchment area of Jeur (Ba) is semi-arid and deciduous mainly with herbaceous species. The study area covers small villages like Waghwadi, and Sasewadi. Most of the people from the region are farmers belonging to various religions; cultivate onion and sugarcane as main crops. They care and rear livestocks for their family maintenance. The farmers use the herbal medicines traditionally to cure their different ailments.

The vegetation of the study area is rich in herbaceous flora, which has great medicinal values. The vegetation has not yet been surveyed for its medicinal aspects. Therefore, a survey was undertaken during 2009 to explore the vegetation. The present work incorporates studies on angiospermic herbaceous and deciduous plants belonging to 27 families embracing 48 genera and 50 species, collected from hills and plains of the village. The information on the collected species were recorded for their medicinal uses by using documents available (Kirtikar and Basu, 1984; Caius, 1986; Trivedi and Sharma, 1986; Kaushik, 1988; Bakhru, 1994; Chaudhury, 1994; Sinha, 1996; Kumar *et al.*, 1997; Bhattacharjee, 1998; Deokar, 1998; Joshi, 2000; Swami Brahamananda, 2000; Auti *et al.*, 2004, Singh and Tyagi, 2006) as well as information was collected from local informants also.

MATERIAL AND METHODS

In order to get information regarding deciduous plants and their medicinal importance the study area was explored during 2009. The authenticity of information was verified by free discussion with local informants. The plants were identified with the help of local flora (Cooke, 1903; Sutaria, 1962; Pradhan and Singh, 1999). Enumeration follows the pattern of binomial, family name, plant part used and their uses for curing diseases. The plants are arranged alphabetically.

ENUMERATION OF COLLECTED PLANTS

1. *Abrus precatorius* Linn. (Fabaceae)
   Plant part used- Seeds, leaves and roots.
   Used in- General tonic, chronic inflammation of eyes, intestinal worms and skin diseases.
2. *Abutilon indicum* (L.) Sweet. (Malvaceae)
   Plant part used- Seeds, leaves and roots.
   Used in- Cough, dyspepsia, piles, leucorrhoea and tuberculosis.
3. *Acacia catechu* (Linn.f) Willd. (Mimosaceae)
   Plant part used- Leaves, bark, wood and inflorescence.
   Used in- Gonorrhoea, sore mouth, chest pains, asthma and cancer. The bark (katha) is astringent, cooling and digestive. The gum has masticating property.
4. *Achyranthes aspera* Linn. (Amaranthaceae)
   Plant part used- Whole plants.
   Used in- Bronchitis, cough, dropsy, dysentery, dyspepsia, vomiting, skin diseases, urinary concretions and pruritus, toothache, bleeding piles, insect bites and in hydrophobia.
5. *Argemone mexicana* Linn. (Papaveraceae)
   Plant part used- Whole plant.
   Used in- Eczema, ulcers, leprosy, dropsy, jaundice and boils.
6. *Asparagus racemosus* Willd. (Liliaceae)
   Plant part used- Roots.
   Used in- Dysentery, tumour and inflammation. It is also recommended in diseases of eye, blood and throat.
7. *Azadirachta indica* A. Juss. (Meliaceae)
   Plant part used- Bark, leaves, seeds and fruits.
   Used in- Burning sensation near the heart, fever, cough, ulcers, inflammation, leprosy, skin diseases, to expel the worms and as diuretic.

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8. *Bacopa monnieri* (Linn.) Penne. (Scrophulariaceae)
Plant part used- Whole plant.
Used in- Bone fracture, in nervous disorders, mental diseases, constipation. It is also used as diuretic, blood purifier, and laxative. Leaf juice is given to infants in bronchitis and in skin diseases, leucoderma and general debility.

9. *Boerhaavia diffusa* Linn. (Nyctaginaceae)
Plant part used- Whole plant.
Used in- Anaemia, swellings, asthma, fever, ascites, jaundice and inflammation of urinary tract, opthalmia and cardiac disorders, muscular pains and to purify the blood, delivery and dropsy.

10. *Cadaba indica* Lam. (Capparidaceae)
Plant part used- Leaves and roots.
Used in- Uterine obstructions.

11. *Calotropis procera* R. Br. (Asclepiadaceae)
Plant part used- Whole plant.
Used in- Skin diseases.

12. *Cardiospermum halicacabum* Linn. (Sapindaceae)
Plant part used- Seeds, leaves and roots.
Used in- Strangury, fever, lumbago, arthritis, otalgia and opthalmodynia, amenorrhea and neuropathy.

13. *Carissa carandus* Linn. (Apocynaceae)
Plant part used- Roots, leaves and fruits.
Used in- Laxative, anthelmintic, appetizer and thermogenic.

14. *Cassia auriculata* Linn. (Caesalpiniaceae)
Plant part used- Bark, roots, leaves and fruits.
Used in- Asthma, chronic fever, dysentery, skin diseases, tumour and urinary discharges.

15. *Cassia tora* Linn. (Caesalpiniaceae)
Plant part used- Leaves and roots.
Used in- Eczema, dermatitis, itchinis, ulcers, cough, fever and intestinal problems.

16. *Ceropegia bulbosa* Roxb. (Asclepiadaceae)
Plant part used- Tuber.
Used in- Colds, eye diseases and to cause sneezing.

17. *Ceropegia tuberosa* Roxb. (Asclepiadaceae)
Plant part used- Whole plant - tubers.
Used in- Diarrhea, dysentery, inflammations, fever, tonic and bowel complaints.

18. *Commelina benghalensis* L. (Commelinaceae)
Plant part used- Whole plant.
Used in- Burns, boils, swellings, leprosy, nervous disorders and erysipelas.

19. *Croton tiglium* L. (Euphorbiaceae)
Plant part used- Seeds.
Used in- Garling, intestinal rumbling, cold, fever, cough, asthma, constipation, calculus, dropsy and ascites.

20. *Cyperus rotundus* L. (Cyperaceae)
Plant part used- Rhizome.
Used in- Cholera, erysipelas, epilepsy, cough, fever, diarrhea, on abscesses and wounds.

21. *Daemia extensa* R. Br. (Asclepiadaceae)
Plant part used- Leaves.
Used in- Asthma, diarrhea, cough and intestinal worms, on swellings and rheumatic joints.

22. *Datura metel* L. (Solanaceae)
Plant part used- Whole plant.
Used in- Asthma, cough, ulcers, fever, skin diseases and in insanity.

23. *Dicrostachys cinerea* W. & A. (Mimosaceae)
Plant part used- Roots.
Used in- Rheumatism, strangury, urinary calculi, renal troubles, diseases of vagina and uterus, retention of urine, pain in joints and eye troubles.

24. *Digera arvensis* Forsk. (Amaranthaceae)
Plant part used- Flowers and seeds.
Used in- Urinary discharge.

25. *Echinops echinatus* Roxb. (Asteraceae)
Plant part used- Roots.
Used in- Wounds in cattle and destroy maggots.

26. *Eclipta alba* Hassk. (Asteraceae)
Plant part used- Whole plant.
Used in- Ulcers and wounds in cattle.

27. *Euphorbia hirta* L. (Euphorbiaceae)
Plant part used- Whole plant.
Used in- Swellings, boils, piles, bronchitis, cough, asthma, vomiting, gonorrhea and colic troubles.

28. *Glossocordia bosvallea* D C. (Asteraceae)
Plant part used- Whole plant.
Used in- Skin diseases.

29. *Hemidesmus indicus* R. Br. (Asclepiadaceae)
Plant part used- Roots.
Used in- Skin diseases, blood purification, fever, loss of appetite, chronic rheumatism, syphilis, gravel, urinary diseases, leucorrhoea, scorpion sting and snake bite.

30. *Jatropha curcas* Linn. (Euphorbiaceae)
Plant part used- Leaves and seeds.
Used in- Ulcers, tumours, scabies, haemorrhoids, skin diseases, rheumatism and paralytic affections.

31. *Kyllinga triceps* Rottb. (Poaceae)
Plant part used- Whole plant.
Used in- Heeling of wounds and regulate body heat and to treat nervous troubles and diseases of blood.

32. *Lantana camara* L. (Verbenaceae)
Plant part used- Whole plant.
Used in- Cuts and wounds.

33. *Phaseolus trilobus* Ait. (Papilionaceae)
Plant part used- Leaves and fruits.
Used in- Snake bite and scorpion sting, eye problems, inflammations, fever, piles, cough and dysentery.

34. *Phyllanthus niruri* L. (Euphorbiaceae)
Plant part used- Whole plant.
Used in- Jaundice or infective hepatitis, diabetes, urinary infections, dropsy intermittent fever, skin diseases and general debility.

35. *Potulaca oleracea* P. (Portulacaceae)
Plant part used- Whole plant.
Used in- Burns, ulcers, urinary disorders, diarrhoea, dysentery, diabetes, scalds, boils, fever, headache, wounds, carbuncles, and cardio-vascular diseases.

36. *Ricinus communis* L. (Euphorbiaceae)
Plant part used- Leaves and seeds.
Used in- Ulcers, soars, skin diseases, boils, rheumatism and headache.

37. *Sida cordifolia* L. (Burm. F) Borss. (Malvaceae)
Plant part used- Whole plant.
Used in- Gonorrhoea, leucorrhoea and nervous diseases.

38. *Solanum xanthocarpum* S. & W. (Solanaceae)
Plant part used- Whole plant.
Used in- Vata, Kapha (cough), helminthiasis, dental caries

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joint pains, constipation, leprosy, skin diseases, hypertension, fever, asthma, bronchitis, strangury, amenorrhoea, dysmenorrhoea, lumbago and haemorrhoids.

39. *Tephrosia purpurea* Pers. (Fabaceae)

Plant part used- Whole plant.

Used in- Inflammation, skin diseases, scrofula, elephantiasis, flatulence, stomach pains, haemorrhoids, asthma, piles, jaundice, bronchitis, anaemia, hepatosplenomegaly, verminosis, strangury, dysenorrhoea, boils, pimples and toothache.

40. *Tinospora cordifolia* (Willd.) Miers. (Menispermaceae)

Plant part used- Whole plant.

Used in- Jaundice, hepatitis and fever, urinary diseases, dyspepsia and flatulence, general debility, sexual impotency, syphilis, gonorrhea, piles and intestinal worms, diarrhea and dysentery.

41. *Tribulus terrestris* Linn. (Zygophyllaceae)

Plant part used- Whole plant - leaves and fruits.

Used in- Menstrual trouble, gonorrhoea, urinary calculi, scalding of urine, strangury, dysurea and vitiated conditions of vata and pitta.

42. *Tridax procumbens* L. (Asteraceae)

Plant part used- Leaves.

Used in- Wounds, boils and in eye diseases.

43. *Triumfetta rhomboidea* Jac. (Tiliaceae)

Plant part used- Leaves, flowers and fruits.

Used in- Gonorrhea.

44. *Tylophora indica* (Burm. f) Merr. (Asclepiadaceae)

Plant part used- Leaves and roots.

Used in- Blood purification, rheumatism, whopping cough, bronchitis, diarrhea, dysentery and fever.

45. *Typha angustata* Chaubard (Typhaceae)

Plant part used- Roots.

Used in- Fever, worms, diarrhea, gonorrhea and indigestion, burns and wounds.

46. *Urginia indica* Kunth. (Liliaceae)

Plant part used- Whole plant.

Used in- Hypertension and to regulate menstrual periods.

47. *Verbascum thapsus* Linn. (Scrophulariaceae)

Plant part used- Leaves, flowers and roots.

Used in- Chest complaints, gout, rheumatism, diarrhea and cough, asthma, pulmonary diseases and bleeding of lungs and bowels.

48. *Vernonea cinerea* (L.) Less. (Asteraceae)

Plant part used- Whole plant.

Used in- Fever, headache, insomnia and as vermifuge.

49. *Xanthium strumarium* L. (Asteraceae)

Plant part used- Whole plant - leaves and fruits.

Used in- Treat skin and bladder infections, stop bleeding from cuts and abrasions, tuberculosis, herpes and small pox.

50. *Ziziphus mauritiana* Lamk. (Rhamnaceae)

Plant part used- Leaves, fruits and roots.

Used in- Cough, biliousness, headache, fever, wounds, boils, diarrhea, indigestion and blood purification.

RESULTS AND DISCUSSION

Phototherapy seems to be one of the remedial measures for the tribals inhabiting the area. Comparison of the data collected with the data already available in the literature shows that the local people including tribal communities have a sound knowledge of the medicinal value of the plants in their area. As the results reported are very interesting, a photochemical work undertaken in at least few plants reported will prove to be added information to literature.

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Medicinal Plants Along the Water Stream of Pradhanpat Water Fall of Deogarh Forest Range in Deogarh District (Orissa)

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The present communication deals with the medicinal flora found along the catchments of the stream of Pradhanpat Water-fall of Deogarh Forest Range in Deogarh District of Orissa. This range is rich with a variety of flora some of which with medicinal values. Survey was conducted by the authors along the stream during 2007-2009 to find out the present status of medicinal flora. A total of 48 medicinal plants belonging to 45 genera and 29 families have been recorded. The medicinal uses by the local inhabitants of these plants have been discussed.

Keywords : Medicinal Plants, Pradhanpat.

INTRODUCTION

In India medicinal plants are used directly in folk remedies or indirectly in modern medicine (Shah, 2008). Medicinal plant diversity and conservation in 21st century is of paramount importance because the loss of floral diversity causes a serious threat not only to the human being but also to the other living organisms besides causing a lot of damage to the environment (Bhattacharjee, 2000, Mahapatra and Choudhury, 2008). If the loss of plants in general and medicinal plants in particular continues along with other plants, then after a few years there will be a death of herbal drugs for primary health care.

Pradhanpt waterfall, a natural waterfall, is 2 Kms away from Deogarh which is the District Head Quarter of Deogarh District of Orissa. The water source of the fall originates 7.0 Kms away from Deogarh town.

Due to continuous availability of water from the stream, there are variety of plants along the stream which also include many medicinal plants. The study area includes the study of flora from the site of origin of the water stream up to the fall *i.e.*, the base of the Pradhanpat Waterfall. Special emphasis has been given on the therapeutic uses of medicinal flora in and around the study area. After declaration of Deogarh as a district with Deogarh town being its Headquarter, the town is extending in all directions touching even the base of the waterfall. Thus, there is an imminent threat to the plants in general and medicinal plants in particular.

MATERIAL AND METHODS

The method of study is based on the collection, identification, preservation and therapeutic uses of plant species. To study and collect plants, field trips were organized along the water stream during 2007-2009. The study of plants has been made starting from the origin of waterfall till it lost its identity near Deogarh town. The catchments include 100 meters towards left and right sides of the water stream. Quadrate method was adopted for floristic study (Mishra, 1968). Each quadrate has a size of 10 m x 10 m. Minimum 2 quadrates were made towards each side keeping a distance of 100 meters between two quadrates. The distance between two quadrates along the water stream was also 100 meters. The samples collected were identified by using their local names with the help of villagers and herbal medicine practitioners locally known as *Kabiraja* & *Vaidya*. The authenticity of identification of the plants were confirmed from standard Flora Books (Haines 1921-25; Saxena and Brahmam 1994-96; Swain and Dash, 2007). The identified species have been preserved in the Department of Botany, Deogarh College, Deogarh.

In total 48 medicinal plants are listed here which are serially arranged as per their alphabetical botanical names, their local names in brackets, their collection number, family and their therapeutic uses (Table 1).

RESULTS AND DISCUSSION

Deogarh Forest Range occupies the largest area of vegetation (60,425.8229 hectares) among all five ranges of

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Table 1 : List of medicinal plants along Pradhanpat water fall with their therapeutic uses.

Sl. No.	Botanical name of the plant with Local name and Collection No.	Family	Therapeutic uses
1.	***Abrus precatorius*** L. (Gunja)-58	Fabaceae	• **Dandruff-** Application of coconut oil cooked with *Abrus precatorius* seeds and *Eclipta alba* leaves once a day for 2 weeks. • **Weak eye sight -** 3g of root powder mixed with 3ml of cow's urine is taken orally once a day for 15-20 days to improve eye sight.
2.	***Achyranthes aspera* L.** (Apamaranga)-26	Amaranthaceae	• **Jaundice-** The roots of *Achyranthes aspera* and *Acacia polycantha* are crushed to make paste. 10 g of the paste is taken with butter milk once a day for 21-30 days to cure Jaundice. • **Dysuria -** 3 g of root powder along with milk is taken two times a day for 3-5 days to cure Dysuria
3.	***Aegle marmelos* L.Corr.** (Bel)-57	Rutaceae	• **Jaundice -** 25 nos. of tender leaves and 21 nos. of black pepper are taken and crushed to make paste. The paste is taken orally with water for 21 days to cure jaundice. • **Dysentery -** The pulp of ripe fruit is taken and to it is added sugar and water. It is taken once a day for 3-5 days to cure dysentery.
4.	***Alstonia scolaris* (L.) R.Br.** (Chhatiana)-18	Apocyneae	• **Ulcers -** A poultice of powdered roasted leaves is used as a local stimulant once a day for 7 days. • **Post partum care-** The leaves of the plant and fresh ginger are taken and crushed to extract the juice. One spoon of juice is taken 2 times a day for 21 days as a remedy for Post partum care.
5.	***Andrographis paniculata* (Burm.F.) Wall ex. Nees** (Bhuinimba)-106	Acanthaceae	• **Improvement of scanty lactation in mother-** 20 ml of decoction of the plant is taken once a day for 15 days to develop Lactation. • **Odema-** A paste is prepared by crushing the whole plant and 5 gm of *zingiber officinale*. The paste is applied on the affected area once a day for 7 days to cure Odema.
6.	***Anogeissus latifolia* (DC.) Bedd.** (Dhaura)-40	Combretaceae	• **Gonorrhoea-** 20 ml of heart wood decoction is taken 2 times a day for 21 days to cure Gonorrhea. • **Otorrhoea-** Mustard oil cooked with heart wood extract is used as ear drop. 1-2 drops is prescribed 2 times a day for 5-7 days to cure Otorrhorea.
7.	***Aspargus racemosus* wild.** (Satabari)-94	Liliaceae	• **Blood dysentery-** Tubers processed with milk is taken 2 times a day for 5-7 days to cure Blood Dysentery. • **Epilepsy-** 10 ml of juice of tubers mixed in one glass of milk taken once a day for 21-30 days to cure Epilepsy.
8.	***Azadirachta indica* A.Juss.** (Neem)-103	Meliaceae	• **Intestinal worms-** A pinch of salt is added to one spoon of fresh juice of leaves and is taken once a day for 3-5 days in empty stomach to expel intestinal worms. • **Jaundice-** One spoon of fresh juice of leaves mixed with honey is taken once a day for 21-30 days to cure Jaundice.
9.	***Bambusa bambos* Voss.** (Kantabaunsa)-42	Poaceae	• **Achene-** Young shoot tip paste is applied once a day for 7-10 days to crue Achenes. • **Cleaning of maggot infected sores-** A poultice of tender leaves is applied on the affected area once a day for 7 days.
10.	***Buchanania lanzan* Spreng.** (Chara)-07	Anacardiaceae	• **Blood dysentery-** 3 g of stem bark powder mixed in one cup of goat milk is taken once a day for 3-5 days to cure Blood dysentery. • **Fever-** Fruit juice is taken 2 times a day for 7 days to cure Fever.
11.	***Butea monosperma* (Lam). Taub.** (Palasa)-35	Fabaceae	• **Helminthiasis-** 3g of seed paste mixed in 1 cup of rice water is taken once a day for 7 days to cure Helminthiasis. • **Leucorrhoea-** In a cup of milk, 5 g of leaf powder and 5 g of candy are mixed together and taken orally once a day for 3 weeks to cure Leucorrhoea.

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12.	***Carea arborea* Roxb.** (Kumbhi)-46	Lecythidaceae	• **Spermatorrhorea-** 20-50 ml of stem bark decoction is taken once a day for 21 days to cure Spermatorrhorea.
13.	***Cartuna regam spinosa* (Thunb.) Turuv.** (Kaleikanta)-105	Rubiaceae	• **Epilepsy-** One gram of seed powder is taken orally followed by one glass of water once a day for 21-30 days to cure Epilepsy. • **Headache-** The root bark paste is applied on the forehead 2 times a day for 2-3 days to cure Headache.
14.	***Cassia fistula* L.** (Sunari)-28	Caesalpiniaceae	• **Jaundice-** 3 g of fruit pulp mixed in 5 ml of fruit juice of *Emblica officinalis* is taken once a day for 3 weeks to cure Jaundice. • **Reheumatic arthritis-** Leaves cooked with mustard oil are rubbed on the affected parts once a day for 20-21 days to cure Rheumatic arthritis.
15.	***Celastrus paniculatus* willd.** (Pingu)-98	Celastraceae	• **Amenorrhoea-** Fresh leaves cooked with 5 g of ghee is taken once a day for 7 days to cure Amenorrhoea. • **Gout-** 20 ml of decoction of the seed is taken once a day for one month to cure Gout.
16.	***Dalbergia sissoo* Roxb. Ex DC.** (Shishu)-38	Fabaceae	• **Any fever-** 50ml of decoction of heart wood is taken once a day for 5-7 days to cure any type of Fever. • **Eczema-** The heart wood oil is applied on the affected parts of the skin two times a day for 12-15 days to cure Eczema.
17.	***Desmodium gangetium* (Lin.) DC.** (Salaparni)-107	Fabaceae	• **Smooth delivery-** The root is crushed to make paste. The paste is mixed with rice washed water and applied on the lower abdomen for Smooth delivery. • **Migraine-** Leaf juice is used as nasal drop when symptoms appear. One drop in each nostril is advised to cure Migraine.
18.	***Diospyrus malabarica* (Desr.) Kostel.** (Mankada kendu)-17	Ebanaceae	• **Burn-** The fruit paste mixed with ghee is applied 2 times a day on the burn areas till it heals. • **Paediatric hiccough-** The paste of flowers/fruits mixed with honey and ghee is taken 2 times a day for 7 days to cure paediatric hiccough.
19.	***Emblica officinalis* Gaertn.** (Anla)-19	Euphorbiaceae	• **Gout-** Fruit juice processed with ghee is taken 2 times a day for 3 weeks to cure Gout. • **Leucorrhoea-** 3 g of fruit powder, 3 g of candy and 3 ml of honey are mixed together and taken once a day for 7 days to cure Leucorrhoea.
20.	***Erythrina variegata* L.** (Paladhua)-74	Fabaceae	• **Helminthiasis-** 5 ml of leaf juice mixed with 3 ml of honey is taken once a day for 5-7 days to cure Helminthiasis. • **Improvement of scanty lactation in mother-** 5 ml of leaf juice mixed with soft pulp of coconut is taken 2 times a day for 21-30 days to develop Lactation in mother.
21.	***Ficus racemosa* L.** (Dimiri)-59	Moraceae	• **Miscarriage-** 50 ml of decoction of fruit mixed with 10 g of rice powder, 5 ml of honey and 10 g of candy is taken together once a day for 7 days to check miscarriage. • **Chronic dysentery-** Steamed tender fruits mixed with curd is taken 2 times a day for 5-7 days to cure Chronic dysentery.
22.	***Ficus hispida* L.f.** (Sasakera, Panidimiri)-61	Moraceae	• **Leucoderma-** 3 g of root powder mixed with 3 g of seed powder of *Psoralea corylifolia* followed by one glass of butter milk is taken once a day for 3 weeks to cure Leucoderma. • **Meno-metrorrhagia-** 10 ml of fruit juice mixed with 3 ml of honey is taken once a day for 21-30 days to cure Meno-metrorrhaagia.
23.	***Flacourtia indica* (Burm.f.) Merr.** (Kanteikoli)-100	Flacourtiaceae	• **Jaundice-** Taking of 5-7 nos. of ripe fruits once a day for 21days to 1 month is advised to cure Jaundice. • **Rheumatic arthritis-** Bark paste cooked with mustard oil is applied 2 times a day for 2-3 weeks to cure Rheumatic arthritis.
24.	***Gardenia gummifera* L.f.** (Ghurudu)-48	Rubiaceae	• **Foul ulcers-** Dusting resin on the affected parts once a day for 7 days kills maggots and prevents maggotification. • **Paediatric diarrhoea during dentition-** Resin is rubbed on the gums to check irritation and 50 g of resin is taken orally once a day for 5-7 days to prevent Paediatric diarrhoea during dentition.

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25.	***Gmelina arborea* Roxb.** (Gambhari)-104	Verbenaceae	• **Urticaria-** Dried ripe fruit processed with milk is taken once a day for 3-5 days to check Urticaria. • **Slackness of breast-** Coconut oil processed with bark is massaged once a day for 3-5 weeks to check Slackness of breast.
26.	***Haldina cordifolia* (Roxb.) Ridsdok.** (Kurum)-52	Rubiaceae	• **Alti** (a type of jaundice)- Root bark paste is applied once a day for 7 days to cure Alti. • **Bruises-** Stem bark paste is applied on the affected parts once a day for 5-7 days to cure Bruises.
27.	***Hemidesmus indicus* R.Br.** (Anantamula)-99	Asclepiadaceae	• **Bronchitis-** Ghee cooked with double quantity of decoction of *Desmodium gangeticum* plant is taken two times a day for 15 days to cure Bronchitis. • **Syphils-** Decoction is prepared by taking the roots and *Tinospora cordifolia* plant. 20 ml of the decoction is taken once a day for 3 weeks orally and it is also applied externally.
28.	***Holarrhena pubescens* wall. Ex G. Don.** (Kurei)-30	Apocynaceae	• **Amoebic dysentery, Blood dysentery-** 50 ml of stem bark decoction is taken once a day for 5-7 days to cure Amoebic and Blood dysentery. • **Bleeding haemorrhoids-** 3 g of ginger powder is mixed in 20 ml of stem bark decoction. It is taken once a day for 21-31 days to cure Bleeding haemorrhoids.
29.	***Ichnocarpus frutescens* (L.) R.Br.** (Sualai)-100	Apocynaceae	• **Diarrhoea-** 1 tea spoon of root paste cr powder is taken 2 times day for 5-7 days to cure Diarrhoea.
30.	***Lannaea coromandelica* (Houtt.) Merr.** (Mai)-56	Anacardiaceae	• **Neck spondylytis-** Stem excudation mixed with *Thespesia populnea* root pounded in cool water is taken as a snuff once a day for 2 weeks to cure Neck spondylytis. • **Filaria-** Leaves ponded with vinegar is taken 2 times a day for 21-30 days to cure Filaria.
31.	***Madhuca indica* Gmel.** (Mahula)-36	Sapotaceae	• **Corneal opacity-** Heart wood paste mixed with honey is taken two times a day for two weeks to cure Corneal opacity. • **Tonic-** Flower cooked with candy, cumin and ghee are taken once a day for 2-3 months to improve Health.
32.	***Mangifera indica* L.** (Amba)-27	Anacardiaceae	• **Diabetes-** 5-10 g powder of tender leaves followed by water is taken once a day for 21-30 days to cure Diabetes. • **Hiccough-** Smoke from burning leaf is inhaled once a day for 3-5 days to check Hiccough.
33.	***Morinda pubescens* Sm. in Rees.** (Aachhu)-51	Rubiaceae	• **Tonic bath-** Bath in infusion of roots once a day for 15-20 days gives better results. • **Summer boils-** The root paste is applied on the boils once a day for 7-10 days to cure them.
34.	***Mucuna puriens* (L.) DC.** (Baidanka)-62	Fabaceae	• **Aphrodisiac-** Seed powder 3-5 g and 5 g of candy mixed in 1 glass of milk is taken once a day to improve Sexual passion. • **Cough and Asthma-** Smoking with the plant once a day for 2 weeks cures Cough and Asthma.
35.	***Nyctanthes arbortristis* L.** (Gangasiuli)-82	Oleaceae	• **Sciatica-** 20-50 ml of leaf decoction is taken once a day for 15 days to check Sciatica. • **Arthritis-** 50 ml of root decoction is taken 3-4 weeks to cure Arthritis.
36.	***Ocimum cannum* Sims.** (Banatulasi)-90	Lamiaceae	• **Conjunctivitis-** Leaf juice is used as eye drop 2 times a day for 7 days to cure Conjunctivitis. • **Acute abscess-** Smearing of leaf paste once a day for 7-15 days cures Abscess.
37.	***Pandanus fascicularis* Lam.** (Kia)-23	Pandanaceae	• **Habitual abortion-** 5 g of root powder mixed with 50 ml of milk is taken once a day for 3-5 days for Habitual abortion. • **Menorrhagia-** 5 g of root paste mixed with 3 g of candy is taken once a day for 15-21 days to cure Menorrhagia

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38.	***Pongamia pinnata* (L.) Pierre.** (Karanja)-37	Fabaceae	• **Diabetes-** 1 g of flower powder mixed in 3 ml of honey is taken once a day for 21-30 days to cure Diabetes. • **Haemorrhoids-** 3 g of Leaves cooked with ghee and sesame oil is applied on the affected area 2 times a day for 21 days to check Haemorrhoids.
39.	***Pterocarpus marsupium* Roxb.** (Bija)-77	Fabaceae	• **Diabetes-** Powder, paste or infusion of heart wood is taken once a day for 3 - 4 weeks to cure Diabetes. • **Gonorrhoea-** 50 ml of heart wood decoction is taken orally for 7-15 days to cure Gonorrhoea.
40.	***Schleichera oleosa* (Lour.) Oken.** (Kusum)-03	Sapindaceae	• **Wound-** The seed paste is applied once a day for 7 days to cure Wounds. • **Scrotal enlargement-** 2.5ml of seed oil mixed in a cup of milk is taken once a day at bed time for 21 days to cure Scrotal enlargement.
41.	***Shorea robusta* Geartn. f.** (Sal)-01	Dipterocarpaceae	• **Anaemia-** 3 g of heart wood powder mixed with 3 ml of honey is taken once a day for 4 weeks to cure Anaemia. • **Goitre-** 5 g of heart wood paste mixed in 10ml of cow's urine is taken once a day in empty stomach in morning for 21-30 days to cure Goitre.
42.	***Strychnos potatorum* L.f.** (Katak)-22	Loganiaceae	• **Gonorrhoea-** 10 g of seed powder mixed in 3 ml of honey followed by one glass of butter milk is taken once a day for 2 weeks to cure Gonorrhoea. • **Erectile dysfunction-** Takings of milk infusion of seed in the night cures Erectile dysfunction.
43.	***Syzygium cumini* (L.) Skeels** (Jamun)-04	Myrtaceae	• **Dysentery with blood discharges-** 60 ml of leaf juice is taken once a day for 5-7 days to cure blood dysentery. • **Diabetes mellitus-** 5-10 g of seed powder is taken once a day for 3 - 4 months to check Diabetes.
44.	***Tectona grandis* L.f.** (Saguan)-102	Verbenaceae	• **Dysuria-** 5 g of root paste and 5 g of candy mixed with 1 glass of goat milk is taken once a day for 7 days to cure Dysuria. • **Miscarriage-** 3 g of seed powder mixed in 1 glass of milk is taken once daily during first month of pregnancy for Miscarriage.
45.	***Terminalia arjuna* (Roxb. ex Dc.) W. & A.** (Arjuna)-02	Combretaceae	• **Heart disease-** Milk decoction of bark is taken once a day for 3-4 weeks to cure Heart disease. • **Acnes-** Bark paste mixed with honey is applied on the affected area once a day for 7 days to cure Acnes.
46.	***Terminalia bellirica* (Gaertn.) Roxb.** (Bahada)-54	Combretaceae	• **Cough-** 10 g of fruit powder mixed with 3 ml of honey is taken 2 times day for 5-7 days to cure Cough. • **Diarrhoea-** Fruit ash mixed with salt is taken 2 times a day for 7 days to cure Diarrhoea.
47.	***Terminalia tomentosa* (DC.).W.& A.** (Asana)-29	Combretaceae	• **Fracture-** 5-10 g of stem bark powder mixed in one glass of milk is taken once a day for 3-4 months to cure Fracture. • **Washing ulcers, wounds-** Decoction of stem bark is used in washing Ulcers and Wounds.
48.	***Wood fordia fruiticosa* Curz.** (Dhatki)-89	Lythraceae	• **Leucorrhoea-** 10 g of flower powder mixed with profuse amount of honey is taken once a day for 2 weeks to cure Leucorrhoea. • **Menorrhagia-** 10 g of flower powder followed by water is taken orally once a day for 21-31 days to cure Menorrhagia.

Deogarh Forest Division. Pradhanpat Reserve Forest covering an area of 10,386.19 hectares is famous since the British period (Mooney, 1950). The forest was very dense with vegetation and rich with medicinal flora. The catchments of Pradhanpat Waterfall was also very thick with vegetation with a large number of medicinal plants. The results of the present study show that at present 48 medicinal plants belonging to 45 genera and 29 families are available along the water stream of Pradhanpat water fall.

The gradual disappearance of species is mainly due to biotic interference. Large number of tourists throng the area through out the year and destroy some of the plants. Deogarh town is the District headquarters of Deogarh which was declared as District in 1994 and the town lies very close (2.0 Km) to the waterfall.The local demand for plants as timber and fuel goes on increasing depleting the flora in general and medicinal flora in particular. Moreover, the constant grazing by cattle affects a lot to the flora. A concise list of the wild

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medicinal flora of the area and their uitilization will provide basic information for further studies aimed at conservation, traditional medicine practice and economic welfare of the rural and tribal population of the district.

The tribal and rural people who primarily depend on the medicinal plants for their healthcare may also benefit from this study. Hence it is the need of the hour to have a proper documentation of the medicinal plants in the study area. Steps are to be taken to preserve and conserve these species.

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Pharmacognostical Studies on *Basella alba* L.

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The present study brings out the pharmacognostical investigations on *Basella alba* L. (Basellaceae) for the first time in this taxon. Anatomical, histochemical colour reactions and phytochemical colour reactions were carried out in leaves, stem and root. The values calculated / data collected could be used for the identification of powdered drug of this taxon.

Keywords : *Basella alba* L. Pharmacognostical studies.

INTRODUCTION

The plant *Basella alba* L. considered under Chenopodiaceae by earlier authors (Kirtikar and Basu, 1933) is presently included under Basellaceae (Mathew, 1991). The family is unigeneric and the genus is unitypic containing a single species *Basella alba* L. syn. *Basella rubra* L. It is distributed throughout India. It is a perennial herb; stem very long, slender, twining to right, succulent, glabrous, much branched; leaves broadly ovate, acute or acuminate, thick, apiculate, entire, base often cordate, narrowed into the petiole; flowers white or red, sessile in lax pedunculate spikes, fruits to the size of pea, red, white or black (Fig. 1A and B). Herb is acrid, sweetish, soporific, narcotic, aphrodisiac, laxative,; improves appetite, useful in leprosy, dysentery and ulcers. The juice of the leaves is a popular application to allay the heat, itching and urticaria arising from dyspepsia.

Paliwal (1967) studied the development of stomata of this plant and identified them to be paracytic and anisocytic. Shantha (2005) conducted pharmacognostical and preliminary phytochemical studies in the leaf of *Basella alba*. As a thorough investigation of this plant has not been still done from the angle of anatomy, histochemistry and phytochemistry, the present study was taken up.

MATERIAL AND METHODS

Anatomical works were carried out in leaf, petiole, stem and root by preparing the peelings and transverse sections. Mature leaves were cleared by using 5% NaOH and chloral hydrate solution, washed in water, stained and mounted in 50% glycerine. Maceration was carried out with stem and root materials following Jeffrey's method (Johansen, 1940). Histochemical colour reactions were done by treating free hand sections with different reagents. Phytochemical tests were done with dried powdered drugs (Fig 1C, D and E).

Table 1 : Quantitative values of foliar epidermis.

Sl. No.	Quantitative values		Abaxial	Adaxial
1.	Epidermal cell size (µm)	(L)	107	100
		(B)	40	50
2.	Epidermal cell / mm²		398	361
3.	Stomatal size (µm)	(L)	35	37
		(B)	30	27
4.	Stomata / mm²		63	49
5.	Stomatal index		14.2	12.21
6.	Palisade Ratio		-	6.35
7.	Vein-Islet Number/mm²		3.75	-
8.	Vein Termination Number/mm²		2.07	-
9.	Stem fibre (µm)	(L)	1389	
		(B)	11	
10.	Stem trachea (µm)	(L)	287	
		(B)	23	

L-Length, B-Breadth.

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Table 2 : Histochemical Analysis.

S.N.	Test for	Chemicals/Reagents used	Expected colour change	Resulted colour		
				Leaf	Stem	Root
1.	Lignin	Sections mounted in 1% neutral aqueous potassium permaganate, washed in water, placed in 2% HCl for 2 mts, washed in water,mounted in dilute ammonia solution	Deep red	Brown (+)	Brown (+)	Brown (+)
2.	Tannins	Sections mounted in 1% solution of ferric chloride	Blue	Very light brown (–)	Very light brown (–)	Very light green(–)
3.	Mucilage	Sections mounted in methylene blue	Blue	Light blue (+)	Light blue(+)	Brown (-)
4.	Starch	Sections mounted in 1% iodine solution	Blue	Green (–)	Blue (++)	Blue (++)
5.	Protein	Sections stained in aqueous solution of picric acid for 24 hrs.	Yellow	Yellow (++)	Yellow (++)	Yellow(++)

++ : Markedly present, + : Moderately present, -: Absent.

Table 3 : Phytochemical Analysis.

S.N.	Test for	Chemicals/Reagents used	Expected colour change	Resulted colour		
				Leaf	Stem	Root
1.	Flavonoids	To the substance added few drops of alcohol, 10% NaOH or ammonia	Dark yellow	Very light yellow(+)	Very light yellow(+)	Dark yellow(++)
2.	Glycosides	Substance mixed with little anthrone, few drops of con. Sulphuric acid added, made as paste, warmed gently over water bath	Dark green	Dark green (++)	Light green (+)	Dark green(++)
3.	Alkaloid	To the substance a little amount of dil. HCl and Meyer's reagent added	White precipitate	Light yellow(-)	White precipitate (++)	Light yellow(-)
4.	Phenol	To the substance few drops of alcohol and ferric chloride solution added	Bluish green or red	Blue(+)	Light brown (-)	Brown (-)
5.	Saponin	Substance vigorously shaken with water	Copious lather	Copious lather(++)	Copious lather(++)	Copious lather (++)
6.	Sugar	To the substance Fehling's solution A and B added.	Red	Dark blue (-)	Light blue (-)	Light blue(-)
7.	Tannins	Substance boiled in water for few minutes, filtered, diluted chloride solution added	White precipitate	Dark brown(-)	Yellowish brown(-)	Dust white(+)
8.	Anthroquin ones	Substance boiled in 10% HCl for few minutes, filtered, cooled, partitioned against equal volume of chloroform, 10% ammonia solution added	Rosy pink	Light brown(-)	Light brown(-)	Light brown(-)

++ : Markedly present, + : Moderately present, -: Absent.

OBSERVATION AND RESULTS

In the intercostal region of abaxial surface of the leaf, the cells are large with undulate cell wall. Stomata are mostly paracytic and very rarely anisocytic (Fig 1F). In costal region the cells are much elongated with straight and thick cell wall. Stomata are rarely present (Fig 2A). In intercostal region of adaxial surface, cells are large with straight wall. Stomata are

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Micro and Macro Morphological Features of *Basella alba*.

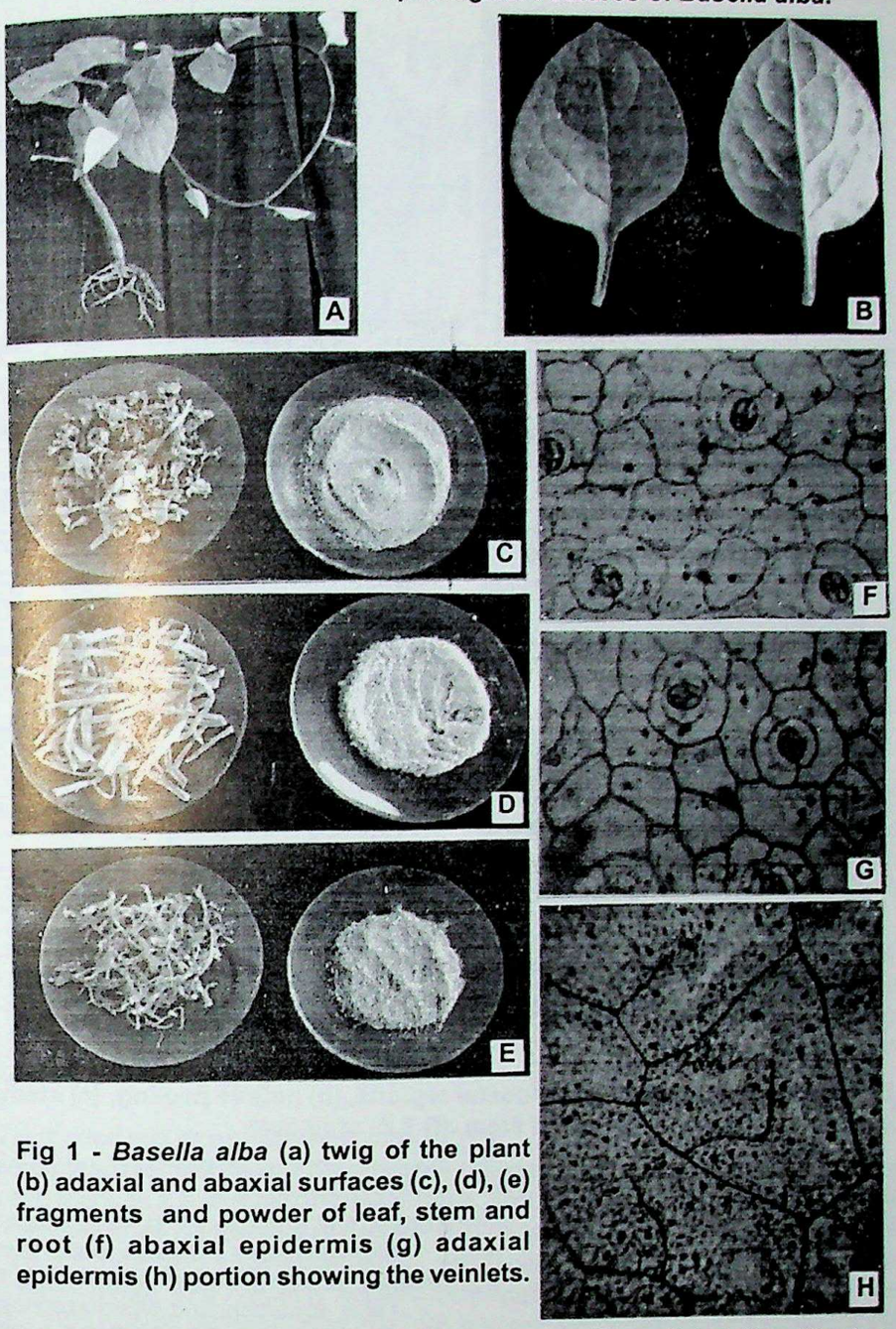

Fig 1 - *Basella alba* (a) twig of the plant (b) adaxial and abaxial surfaces (c), (d), (e) fragments and powder of leaf, stem and root (f) abaxial epidermis (g) adaxial epidermis (h) portion showing the veinlets.

paracytic with a few giant stomata. In costal region cells are much different from the intercostal region. Cell wall is slightly curved and much elongated. Mid vein gives 5-10 lateral branches which further sub-divide to give rise to branch-lets. Mid-vein and lateral veins are deep red in colour. Vein-islets are polygonal and very large having 1 or 2 vein endings. (Fig 1H).

Cells of petiole peelings are much elongated with straight wall and oriented in the direction of longitudinal axis of cell. (Fig 2B). In stem peeling the cells are much elongated and narrow. Stomata are rarely seen (Fig 2C).

In the T.S. of stem vascular bundles are arranged in two rows; outer row formed of 15-20 small bundles and inner row 5 - 8 large bundles (Fig. 2E). T.S. of primary structure of the root shows four patches of xylem and phloem in radial arrangement each patch containing two rows (Fig. 2D).

The petiole in its outline is omega shaped with two small wings (Fig 2F). The other anatomical features are given in Table 1. The presence/absence of different cell constituents identified by histochemical and phytochemical tests are shown in Table 2 and 3.

DISCUSSION

Powdered drugs of many plants are now available as plant products. In such cases, the study of cellular characters of the drugs and the phytochemical tests of their constituents are inevitable (Iyengar, 1974; Iyengar and Nayak, 1975). Moreover, these days the adulteration of drugs is often found to be a deliberate act. Drugs are viewed with great suspicion just because properly identified crude drugs donot seem to be used in their preparations (Desh and Bedi, 1967, 1968; Yaganarasimhan *et al.* 1974). Hence, the need for evolving

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Anatomical features of *Basella alba*

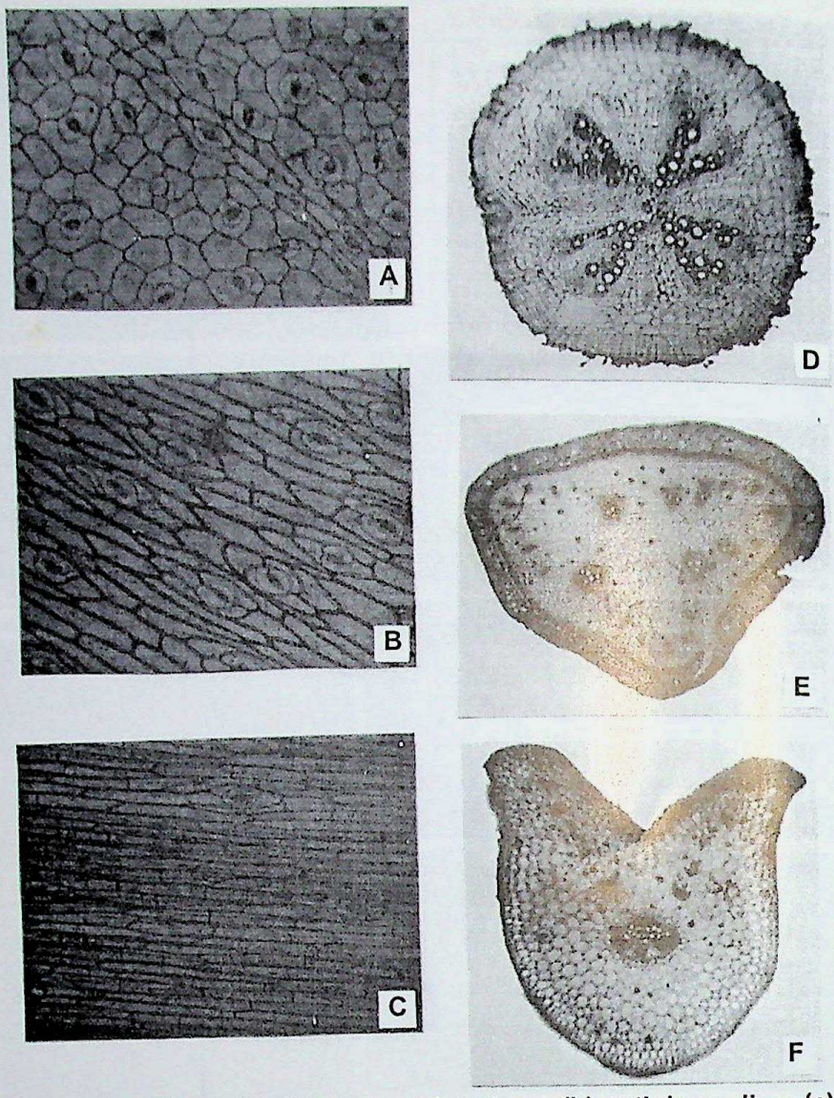

Fig 2 - *Basella alba* (a) Leaf peeling - Costal and intercostal regions, (b) petiole peeling, (c) stem peeling, (d) T.S. of root, (e) T.S. of stem, (f) T.S. of petiole.

criteria for standard samples of crude drugs has became very important in pharmacognosy. A combination of characters such as epidermal cell size, stomatal size, their distribution, stomatal index, presence or absence of hairs, their types, palisade ratio, venation pattern, vein-islet numbers, vein-let termination number are found to be very significant micro- morphological characters in the identification of crude drugs (Krishnamurthy and Sundaram, 1967, Krishnamurthy and Kannabiran, 1970). In the light of above, the anatomical, histochemical and photochemical values for leaf, stem and root drugs of *Basella alba* could be successfully used for the identification of powdered drug of this taxon.

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Indigenous Phyto-Therapy for Gastro-Intestinal Disorders Among Tribals of Dhenkanal District, Orissa

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A study on herbal remedies for gastro-intestinal disorders among the tribals of Dhenkanal district was conducted during the year 2007 - 08. Twenty plant species belonging to 19 genera and 18 families were enumerated. The traditional communities mainly depend on these locally available plant species for treatment of the diseases utilizing their inherited ethno-medicinal knowledge. The phyto-remedial measures of plant species is based on the traditional tools and technology of the ethnic people but still needs further elaborated studies.

Keywords : Dhenkanal district, Dehuri, Ethno-Medicinal Knowledge, Phyto-remedial Measures.

INTRODUCTION

In Orissa, 62 communities have been recognised as schedule tribes. They are unevenly distributed in different districts. Dhenkanal is one of the centrally located districts (85° 58' to 86°2' E and 20 °29' to 21° 11'N), spreading over an area of 4595 sq. km. (Fig 1). The tribals belonging to Santals, Sabara, Saura, Majhi, Kolha and Munda communities constitute 12.79 % of the total population of the district.

The district is divided into three natural regions *viz*, the southern hilly regions, the river valley and the northern hilly regions. The area experiences a sub-tropical moist climate. It is divided into two climate zones *viz*, the northern region which is less moist and the southern and south-east region, which are comparatively more moist. The humidity content varies from 31 to 88%. The total annual rainfall during 2007-08 was 1403.5 mm. Most parts of the district are covered with dense forests with long range of hills. Therefore, the district is known as "The Home of Elephants and Tigers" for the country.

The tribal communities mostly inhabit in remote areas with limited access to the civilized world. They have been depending primarily on herbal medicines practiced by the traditional healers for treatment of common diseases. A number of plant species known to them have been used in the treatment of gastro-intestinal disorders, such as diarrhoea, dysentery, dispepsia and constipation.

Ethno-medico-botanical studies in this part of Orissa is rare. Studies in other parts of Orissa have been conducted by Brahmam and Saxena (1990), Das and Misra (1996) and Girach *et al.* (1999, 2001). Behera and Misra (2005), Behera *et al.* (2006), Mahapatra and Sahoo (2008), Acharya *et al* (2009). Rao *et al.* (2008) have however reported some less known medicinal plants used by indigenous communities from different parts of Dhenkanal district, Orissa.

The present study was conducted with an objective of recording data pertaining to the ethno-medicinal uses of plants by the indigenous communities of Dhenkanal district of Orissa for treatment of various gastro-intestinal disorders.

MATERIAL AND METHODS

The study was carried out during 2007-08 covering Deogaon, Joranda, Kapilas and Bhuban area of north and north-eastern hilly regions of Dhenkanal forest divisions. A close association was maintained with the traditional practitioners to record their indigenous knowledge system on primary healthcare. The medicinal doses prepared from plants and their administration were observed closely. A general survey method with well planned questionnaire was followed for the collection of ethno-medicinal information from the traditional healers called "dehuri". Several field trips were conducted to interview the herbal medicinemen and local elderly persons to enquire about the success of traditional medicines. A cross check was followed with the Baidya's to know the efficacy of the herbal drugs. The plants used by the tribals were collected, identified with help of the local flora (Saxena and Brahmam, 1994-96) and herbarium specimen were prepared and preserved in the department of Botany, Ravenshaw University, Cuttack.

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RESULTS AND DISCUSSION

Ethno-medicinal observations on 20 plant species belonging to 19 genera and 18 families are reported during the investigation in the tribal areas of Dhenkanal district. The plant species with family names are arranged in alphabetical order with locality, local name Oriya name followed by medicinal doses for the treatment of gastro-intestinal disorders emphasizing on dysentery and diarrhoea.

The data collected on medicinal uses of plants during investigation are enumerated below:

The abbreviations used in the text are : locality (Loc) Local name (LN) Oriya name (O).

1. *Achyranthes aspera* L. (**Amaranthaceae**)
   Loc: Joranda; Apamargo (LN); Apamaranga (O)
   10 gms of root paste alongwith 2 to 4 black pepper is taken thrice daily to cure diarrhoea and dysentery.
2. *Aegle marmelos* (L). Cor. (**Rutaceae**)
   Loc: Deogaon; Belo, Baelo (LN); Bela (O).
   Seven leaves with one teaspoon powder of *Cuminum cumini* is administered twice daily in empty stomach for 5 days to cure dysentery of children.
3. *Ageratum conyzoides* L. (**Asteraceae**)
   Loc: Deogaon; Kapilas; Bok sunga (L.N.) Pokasunga (O).
   One to two teaspoonful of fresh leaf juice is taken with a pinch of black pepper powder 2 to 3 times in empty stomach daily to stop dysentery.
4. *Asparagus racemosus* Willd. (**Liliaceae**)
   Loc: Joranda; Atkir, Chhataori (L.N.) Satawari, Satamuli (O).
   Roots are soaked overnight in plenty of water. Then crushed and squeezed with a piece of cloth to extract juice. One teaspoon of this extract along with sugar candy is taken in empty stomach thrice daily for 3 days to cure dysentery.
5. *Careya arborea* Roxb. (**Lecythidaceae**)
   Loc: Joranda; Bhuban, Ashadhua (LN & O)
   One tablespoon of fresh bark juice with equal amount of raw cow milk is taken in empty stomach thrice daily to cure diarrhoea.
6. *Cyperus rotundus* L. (**Cyperaceae**)
   Loc: Deogaon, Joranda; Mutha, Motha (LN and O).
   (1.) One teaspoon of juice extracted from fresh leaves is taken with little amount of sugar candy thrice daily in empty stomach to cure diarrhoea.
   (2.) One teaspoon of fresh root juice is taken twice daily in empty stomach for the treatment of diarrhoea.
7. *Cynodon dactylon* (L) Pers. (**Poaceae**)
   Loc: Bhuban, Deogaon, Joranda; Dhubbo (LN); Dubaghas (O).
   One teaspoon of fresh juice of entire plant is taken in empty stomach thrice daily for treatment of dysentery.
8. *Ficus benghalensis* L. var. *benghalensis* (**Moraceae**)
   Loc: Kapilas, Joranda, Bhuban; Baro (LN), Batobrukshya (O).
   Paste of prop roots with rice washed water is taken in empty stomach thrice daily for treatment of blood dysentery.
9. *Ficus racemosa* L. syn *F. glomerata* Roxb (**Moraceae**)
   Loc: Kapilas, Joranda, Bhuban; Loa (LN), Dimiri (O).
   Stem bark is soaked in water for 2-3 hours, then crushed and squeezed to extract juice. 5 drops of this juice mixed with mother's milk is given to the child thrice daily to cure diarrhea.
10. *Hemidesmus indicus* (L) R. Br. (**Asclepiadaceae**)
    Loc: Joranda; Deogaon; Guopaan (L.N.), Dudhalai, Ananta muli (O).
    Fresh roots (approximately 100 gms) were grinded and made into paste. About 10 gms of this paste is taken with 2-3 black pepper thrice daily to cure diarrhoea of children.
11. *Holarrhena pubescens* (Buc.-Ham.) Wall.Ex. G. Don. syn *H. antidysenterica* auct. non (Roth) Wall. Ex. Dc (**Apocynaceae**)
    Loc: Kapilas, Bhuban; Kulchi (LN), Indra jal (O).
    (1) Seed paste is given with water for curing diarrhoea.
    (2) Seed powder with black pepper is administered with mother's milk to cure diarrhoea of children.
12. *Ipomoea mauritiana* Jacq. Syn. *I. digitata* auct. non L (**Convolvulaceae**)
    Loc: Bhuban; Bhuin Kokharu (LN & O).
    Fresh rhizome is crushed and given with water in empty stomach thrice daily to get releif blood dysentery.
13. *Kalanchoe pinnata* (Lam.) Pers. Syn. *Bryophyllum calycinum Salisb.* (**Crasssulaceae**)
    Loc: Kapilas, Bhuban; Amarpoi (LN & O).
    One teaspoonful of fresh leaf juice alongwith 7 black pepper is administered twice daily in empty stomach for 3 days to cure diarrhoea and dysentery.
14. *Madhuca indica* Gmel. Syn. *Bassia latifolia* Roxb. (**Sapotaceae**)
    Loc: Kapilas, Mahua (LN), Mahula (O).
    Decoction of equal amounts of bark of *Madhuca indica* (Mahula), *Mangifera indica* (Mango), and *Syzygium cumini* (Black Berry) is prepared. Two teaspoons of this decoction is taken with honey thrice daily in empty stomach for 4-5 days to cure blood diarrhoea and dysentery.
15. *Mangifera indica* L. (**Anacardiaceae**)
    Loc: Kapilas, Bhuban; Amba (LN & O).
    (1) Two teaspoons of crushed young leaves are taken with water thrice daily in empty stomach to cure diarrhoea.
    (2) Two teaspoon of juice prepared from stem bark is taken with water thrice daily in empty stomach to cure dysentery.
16. *Momordica charantia* L. (**Cucurbitaceae**)
    Loc: Deogaon, Joranda; Karla (LN), Kalara (O).
    One teaspoon of fresh leaf juice alongwith raw cow milk with a pinch of salt is taken twice daily in empty stomach to get relief from constipation.
17. *Phyllanthus fraternus* Webster syn.*P. niriri* auct. non. L (**Euphorbiaceae**)
    Loc: Joranda, Bhuban; Bhuianla (LN), Badi aonla, Bar amla (O).
    One teaspoon of root powder with a pinch of black pepper alongwith water is given to check amoebic diarrhoea and dysentery of children.

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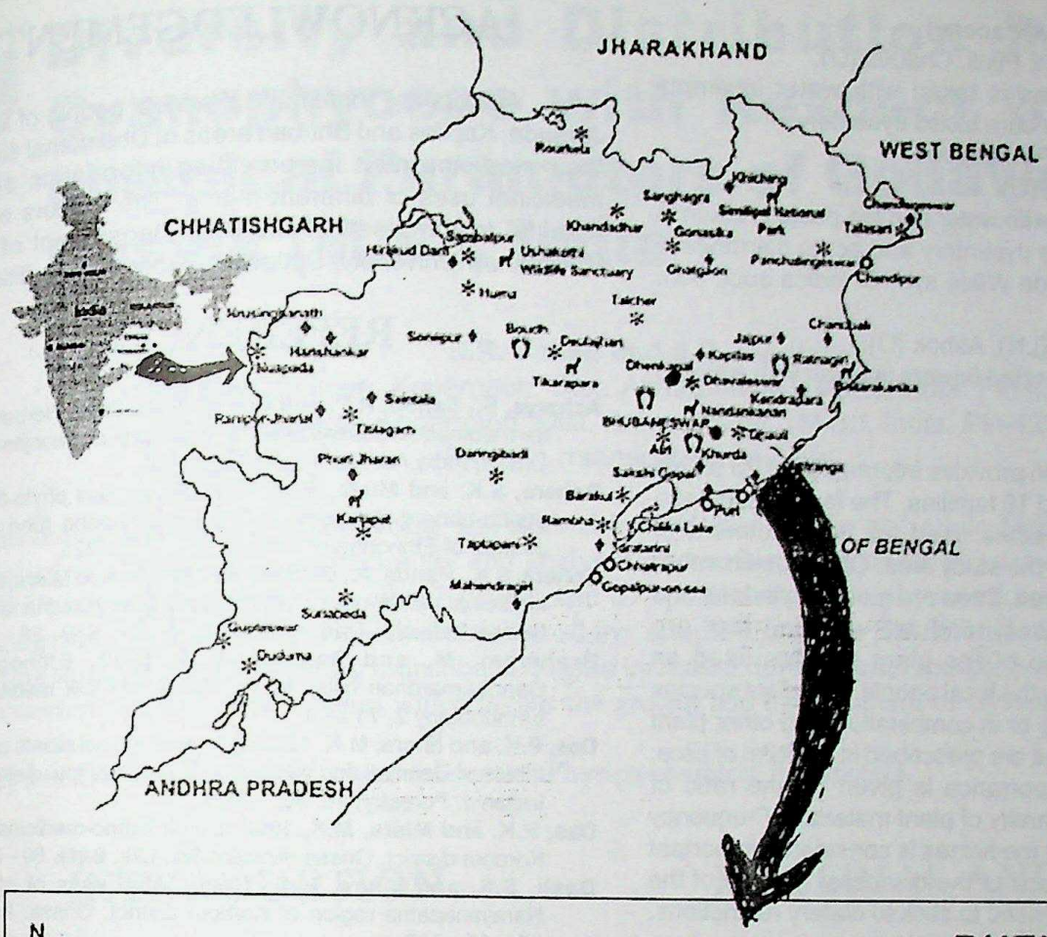

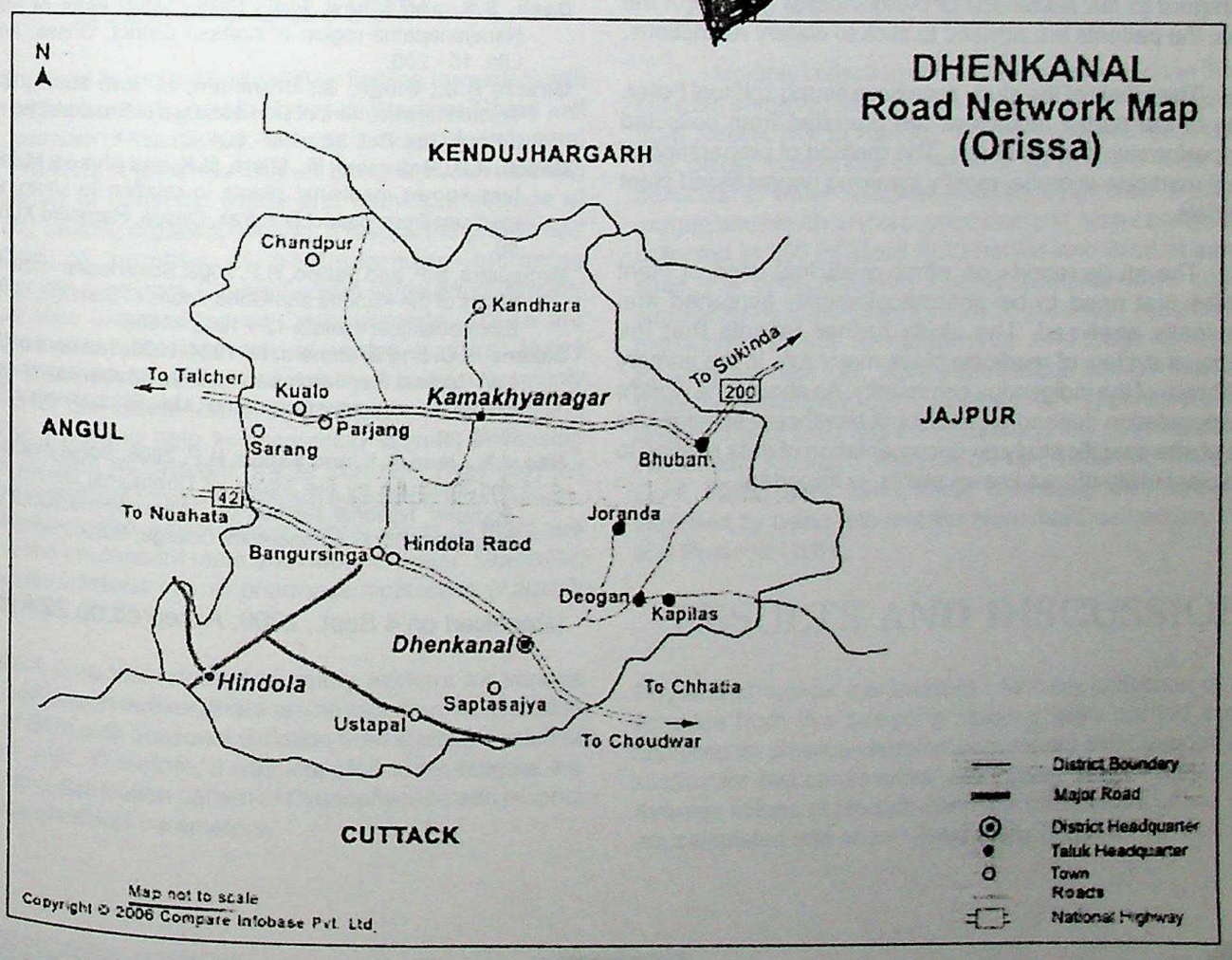

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18. *Psidium guajava* L. (**Myrtaceae**)
    Loc: Bhuban, Deogaon; Pijuli, Chaulia (O).
    Paste of tender leaves is taken with water in empty stomach thrice daily to cure blood dysentery.
19. *Punica granatum* L. (**Punicaceae**)
    Loc: Bhuban, Dalimba (O).
    Young fruit is crushed with water and the paste is taken in empty stomach to cure dysentery and acute diarrhoea.
20. *Saraca asoca* (Roxb) de Wilde syn. *S. indica* auct. non. L. (**Caesalpiniaceae**)
    Loc: Kapilas; Oshoko (LN), Ashok (O).
    About 10 gms of macerated flowers is taken with water to cure blood dysentery.

Present investigation provides information of 20 plants belonging to 19 genera and 18 families. The family Moraceae contributes maximum species used for the treatment of diarrhoea and dysentery in the study area. Of the total number of 20 plant species described, three are monocotyledons and seventeen are dicotyledons, nine are wild and four are cultivated species. Some of the plant species used as medicines are cultivated by the local people. The plant species are used either individually or in combination with other plant parts. Most of the medicines are prescribed in the form of juice, decoction and paste. Importance is given for the ratio of different elements and quantity of plant materials. Frequency of administration of herbal medicines is considered important with regard to the acuteness of the diseases. In most of the cases, the patients are advised to stick to diatary restrictions.

The tribals of the study area have strong spiritual belief. Some of the herbal medicines are prepared from collected after performing certain rituals. The method of preparation of herbal medicine is crude, mostly involving unprocessed plant materials.

The study reports on ethno-medicinal uses of plant species that need to be pharmacologically screened and chemically analysed. The study further reveals that the traditional system of medicine plays major role in the primary healthcare of the indigenous community. As about 80% world's rural population depend on traditional healthcare system, this type of site-specific study on documentation of data relating to medicinal utility of less known plants, is important.

ACKNOWLEDGEMENT

Authors are thankful to the tribal people of Deogaon, Joranda, Kapilas and Bhuban areas of Dhenkanal district and their medicine men for providing information about the medicinal uses of different plants. The authors are much thankful to the Head of the P.G. Department of Botany, Ravenshaw University, Cuttack for laboratory facilities.

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Diversity and Distribution Pattern of Cyanophyceae with Respect to Physico-chemical Parameters of Godavari River at Nashik (M.S.), India

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Distribution pattern of Cyanophyceae was studied from Godavari river at Nashik. Five sampling sites were selected for collection of water samples and analysed at monthly intervals for physico-chemical parameters and Cyanophycean studies. In all, 17 genera and 27 species of Cyanophycean forms were encountered during study period with seasonal fluctuation at all five sampling stations. The flora was found to be abundant during summer, declined during monsoon and again increased during winter season. Some physico-chemical parameters showed higher values which favour the growth and development of Cyanophyceae members.

Keywords : Godavari river, Cyanophyceae, Physico-chemical Parameters.

INTRODUCTION

Godavari is second largest river flowing through South India. It originates at Trymbakeshwar in Western Ghats just 30 Km. upstream of Nashik city. Flowing through Maharashtra, Andhra Pradesh, it joins the Bay of Bengal. River receives huge quantity of domestic waste and municipal sewage of Nashik city causing organic pollution. It results the growth and population of number of phytoplankton including Cyanophyceae members. Cyanophycean algae are commonly called as blue green algae or Cyanobacteria. These are ubiquitous and abound various water bodies. Many heterocystous BGA fix atmospheric nitrogen and so commonly are useful for rice cultivation. They also grow well on alkaline and saline soils and help in decreasing salinity, increasing nitrogen as well as organic matter content. They play an important autotrophic role and provide food to aquatic animals. They are also used as fertilizers, while some of them are imparting the unpleasant taste and odour to water. Moreover, some Cyanobacteria are of pharmaceutical value (Kaushik and Chauhan, 2009).

BGA flora was studied by many workers for various water reservoirs. However, there are no reports on distribution pattern of BGA with seasonal variation from starting stretch of Godavari river. Therefore, it was intended to investigate the diversity and distribution pattern of Cyanophyceae with respect to physico-chemical parameters.

MATERIAL AND METHODS

Monthly collection of water samples was done from five different sampling stations covering the 30 km stretch of river from Nashik city to Nandur- Madhyameshwar pick up weir. The plastic containers of two liter sized were used for the collection of water samples, while for estimation of dissolved oxygen and bio-chemical oxygen demand, water samples were collected in 250 ml sized BOD bottles and fixed at sampling sites. For phytoplankton studies samples were collected separately by using plankton net 25 meshes / linear inch and preserved in 4% formaldehyde and Lugol's solution. Parameters like pH, light penetration, temperature were detected at sampling sites, while remaining parameters were analyzed after reaching the laboratory. For physico-chemical parameters standard methods (APHA, 1985 and Trivedy and Goel, 1986) were used, while Cyanophycean forms were identified by using relevant literature like Desikachary (1969) and Prescott (1954).

RESULTS AND DISCUSSION

Physico-chemical parameters : Monthly collection of water samples from five sampling stations were carried out and analysed for physico-chemical parameters and Cyanophycean studies for two consecutive years (Aug. 2003 - July. 2005). Average values of physico-chemical parameters of two years are calculated and shown graphically (Figs. 1 - 4).

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Minimum and maximum average light penetration values recorded were 32.59 cm and 73.6 cm at station S4 and S5 respectively. At station S5 minimum (20.32 °C) and at S3 maximum (21.73 °C) average water temperature values were recorded which were found within permissible limits suggested by WHO. *i.e.*, 40°C.

Minimum average EC value (0.63 m mhos/cm) was recorded at station S1 while, maximum at station S4. (0.89 m mhos/cm). Average pH values at all stations showed that river water was alkaline throughout the investigation period. Higher average chloride value was recorded at station S4 (146.5 mg/L) and lower value was recorded at station S1 (109.8 mg/L).

Average nitrate values showed that at station S1 and S5 minimum (0.56 mg/L) and at station S3 maximum (0.82 mg/L) values were recorded.). Low average phosphate values were found at station S1 (0.78 mg/L) and maximum at station S2 (0.95 mg/L).

Minimum average potassium value was recorded at station S5 (1.12 mg/L) and maximum at station S2 and S3 (1.22mg/L). Minimum average value of alkalinity was found at station S1 (84.71 mg/L) and maximum at station S2 (124. mg/L).

Hardness showed lower average values at station S1 (75.77mg/L) and higher at station S4 (130.6 mg/L). Minimum average BOD value recorded was (1.64mg/L) at station S1 and maximum (18.37mg/L) at station S2. Minimum and maximum average free CO_2 values were recorded at station S1 (7.92 mg/L) and S3 (13.2 mg/L) respectively. An average value of dissolved oxygen was found maximum at station S1 (6.29 mg/L) and minimum at station S2 (5.07 mg/L). It was

Table 1 : Cyanophycean algae encountered during investigation period at different sampling stations of Godavari river (August 2003 - July 2005).

Sr. No.	Name of Algae	Stations				
		1	2	3	4	5
1.	*Microcystis aeruginosa* Kuetz.	+	-	-	+	+
2.	*Merismopedia punctata* Meyen	+	-	+	-	+
3.	*Gleocapsa pontanata* Nag.	+	-	+	-	-
4.	*Chroococcus minor* (Kuetz.) Naegeli	-	-	-	+	-
5.	*Chrococcus minutus* (Kuetz.) Nag.	+	-	+	+	-
6.	*Chrococcus turgidus* (Kils.) Nag.	-	+	-	+	+
7.	*Aphanocapsa regularis* Carm.	+	-	+	-	+
8.	*Amphinothece nidulans* P. Richter	+	-	+	+	+
9.	*Gomphospaeria aponina* Kuetz.	+	+	-	+	+
10.	*Dactylococcopsis raphidioides* Hansg.	+	+	-	-	+
11.	*Spirulina laxissima* G. S. West	+	+	-	-	-
12.	*Spirulina princeips* W. and G.S.West	-	+	-	+	+
13.	*Spirulina major* Kuetz.	-	-	+	-	-
14.	*Oscillatoria chalybea* (Martens) Gom	+	-	-	-	+
15.	*Oscillatoria formosa* Borb	-	-	+	+	-
16.	*Oscillatoria anguina* (Bory) Gomont	-	-	-	-	+
17.	*Oscillatoria limosa* Ag.	-	-	-	+	-
18.	*Lyngbya contorta* Lemm.	+	-	-	+	-
19.	*Lyngbya birgei* G.M.Smith	-	+	-	-	-
20.	*Arthrospira massartii* Kuffarth.	-	+	+	-	+
21.	*Arthrospira khanne* Drougeland and Strickland	-	+	-	-	+
22.	*Nostoc commune* Vaucher ex Born. e t Flah.	-	-	+	-	-
23.	*Anabaena orientalis* Dixit.	-	+	-	-	+
24.	*Anabaena spiroides* Klebs.	-	+	+	-	+
25.	*Tolypothrix distorta* Kuetz.	+	-	-	+	-
26.	*Aulosira bombayensis* Gonzalves	-	+	-	+	+
27.	*Rivularia aquatica* De Wilde	+	+	-	-	-

Key : + = present; – = absent.

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Graphical presentation of physico-chemical parameters:

Fig. 1: Average values of light penetration, water temperature and pH during the study period at five sampling stations.

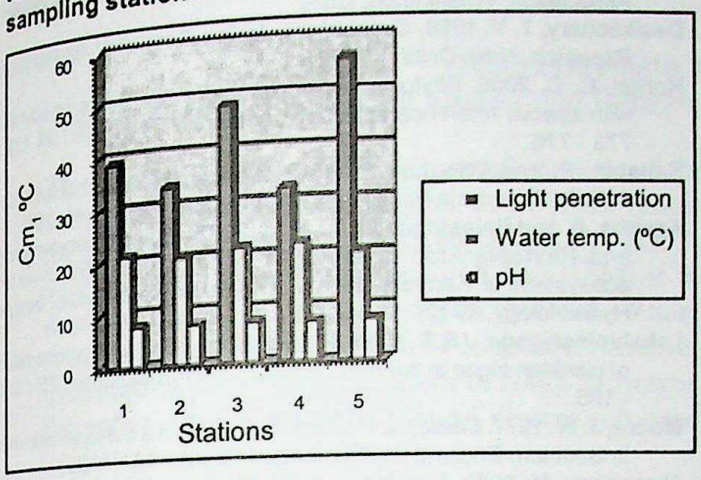


Fig. 2: Average values of chloride, alkalinity and hardness during the study period.

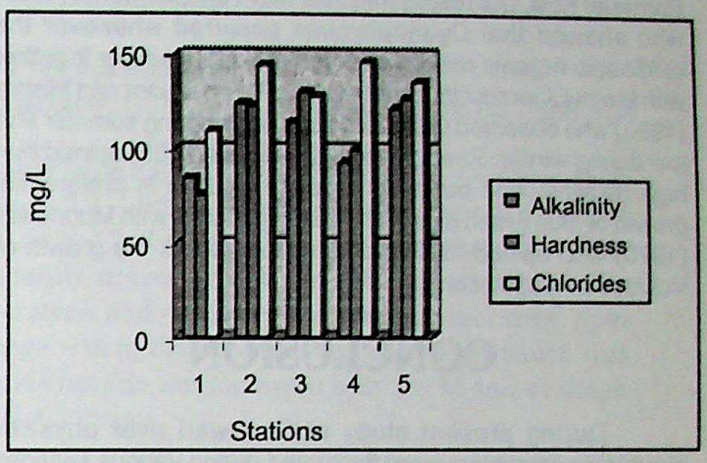


Fig. 3: Average values of E. C., nitrates, phosphates and potassium five sampling stations during the study period.

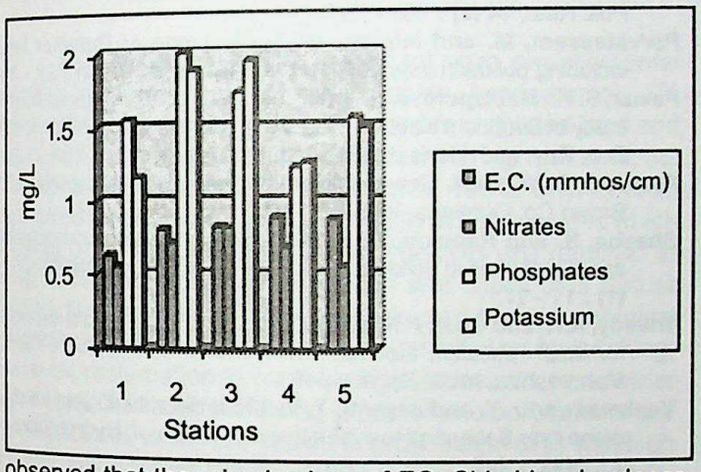
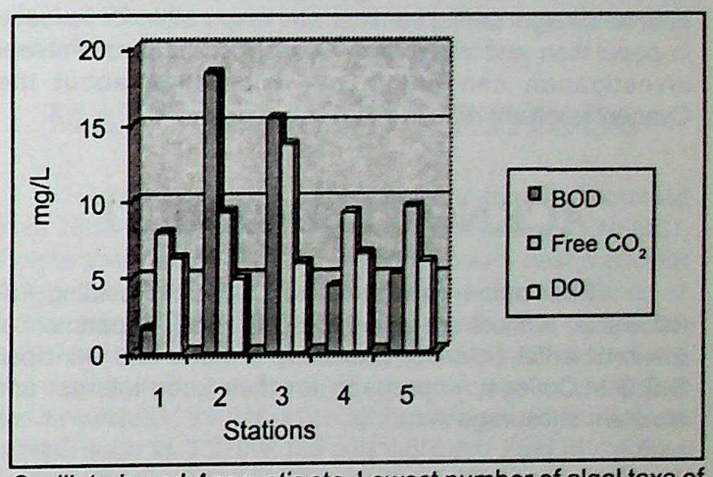


Fig. 4: Average values of BOD, Free CO_2 and DO at five sampling stations during the study period.



observed that the raised values of EC, Chlorides, hardness, alkalinity, BOD, free CO_2, while low values of pH and DO at certain stations during summer season showed the polluted nature of the river water.

Free CO_2 showed higher values during summer season. It was declined at certain stations and was due to high temperature, low dissolution of oxygen, and high organic matter, while more during monsoon was due to low temperature and flood condition. Our result coincides with Venkateswarlu and Jayanti (1968).

Cyanophyceae : This class was represented by 17 genera and 27 species (Table 1) belonging to orders Chroococcolales and Nostocales. The commonly recorded genera were *Oscillatoria, Anacystis, Anabaena, Lyngbya, Spirulina* etc. During summer more number was recorded and might be due to availability of more free CO_2, sunlight, phosphate and nitrate concentration. Our results correlate with Moore (1977). The common pollution tolerant genera recorded during present study were *Lyngbya, Spirulina, Anabaena, Microcystis, Oscillatoria* and *Anacystis* etc. Lowest number of algal taxa of BGA was recorded during monsoon months particularly in months of July, August and September. Lowest number might be attributed to high water velocity, turbidity and dilution of nutrients due to rain. Raised values in temperature, low water velocity and more nutrition with bright sunlight increased the algal population during summer. According to Pawar *et al.* (2006), high organic matter, high temperature and low DO favours the growth of blue green algae.

Maximum development of BGA was reached during the months of March, June and December, while minimum was found in April and October. Cyanophycean forms started to increase after April and by the end of June they showed clear dominance. Our results coincide with Lakshminarayana (1965 b) who opined that abundant growth of blue green algae was due to high values of temperature, high pH, dissolved organic matter, more nitrates and phosphates. During study period, maximum algal population was encountered during late winter and summer seasons which matches with the results of Kavitha (2007).

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According to Harilal (2005) nitrate is positively influences the growth and distribution of members of Cyanophyceae which correlates with our results. Maximum density of Cyanophyceae was reported by Pandey *et al* (1995) from river Kosi. Our results matches with Venkateswarlu, (1969) who showed that Cyanophyceae occurred whenever the oxidisable organic matter was high and DO was low together with low pH. Our results correlate with Parvateesam and Mishra (1993) who observed Cyanophycean peak during summer and low during winter. Sheeba and Ramanujan (2005) opined that high alkalinity and buffering capacity resulted in preferential growth of blue green algae. Our result matches with Munnawar (1970) who opined that alkaline water favours the growth of Volvocales population.

CONCLUSION

During present study of Godawari river physico-chemical parameters were fluctuated during various seasons of the year. River water showed high values of chlorides, alkalinity, hardness, BOD and low values of pH and DO which indicate the organic pollution of river. River water was always alkaline and it favours the growth of Cyanophycean flora. Intense sunlight during summer was responsible for increase in population and diversity of Cyanophycean taxa. Present investigation can enrich our knowledge about the Cyanophycean flora.

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Response of *Oscillatoria willei* to Saline Stress

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The effect of NaCl on the biochemical contents of *Oscillatoria willei* BDU 141621 was studied. *Oscillatoria* was subjected to different concentrations of NaCl ranging from 0.2 – 0.8 M, besides control over a period of 30 days. With a moderate concentrations (0.2 and 0.4M) of NaCl, the total chlorophyll contents increased, while at higher concentrations (0.6 and 0.8M) it was sharply decreased. The protein contents gradually decreased in all the concentrations of NaCl. While β-carotene and carbohydrate contents increased upto 0.6 M and thereafter declined. The most distinctive change was in the accumulation of proline which was increased in all the concentrations of NaCl. However glycine betaine was increased upto 0.6 M and declined thereafter. *Oscillatoria willei* showed diverse response to NaCl stress.

Keywords : *Oscillatoria*, NaCl, β-carotene, Proline, Glycine betaine.

INTRODUCTION

Salinity is one of the important agro environmental problem in the arid and semiarid regions. Approximately 7% of the worlds land area, 20% of the worlds cultivated land and nearly half of the irrigated land is affected with high salt contents (Rhoades and Loveday, 1990, Szabolcs, 1994). Salinity is an undesired threat to crop yield, especially in the countries which depend on irrigational agriculture. Attempts are required to bring back such areas under plough and check their further spread. Normally, such lands are reclaimed through chemical amendments like application of gypsum followed by leaching. However, reclamation through biological means is yet another approach. The most promising group of microorganisms are cyanobacteria, which are known to grow well in soils with high pH and salt content (Singh, 1961; Subhashini and Kaushik, 1982). Cyanobacteria offer an economically attractive and ecologically sound alternative to chemical fertilizers for realizing the ultimate goal of increased productivity, especially in rice cultivation. Since cyanobacteria can both photosynthesise and fix nitrogen. These abilities, together with great adaptability to various soil types make them ubiquitous. Because of its implied agricultural potential in the biological reclamation of salt affected soils, it has gained lots of importance and also attracted number of research workers (Singh, 1950, 1961; Thomas, 1978; Kaushik and Venkataraman, 1982). Normally salinity in agricultural land ranges from 0.015% - 0.21% (Mishra and Srivastava 2003; Goyal and Srivastava 2003). But in adverse conditions the algae with high salt tolerance may be useful in the agricultural fields. Hence deeper understanding of the physiology of cyanobacteria becomes essential for the successful development of algalization technology especially on salt affected soils. Keeping this in view, the higher concentrations of NaCl were chosen for the study.

The present investigation is an attempt to evaluate the effect of different concentrations of NaCl on the total chlorophyll and biochemical contents of *Oscillatoria willei*.

MATERIAL AND METHODS

Oscillatoria willei BDU 141621 is a marine alga obtained from National Facility for Marine Cyanobacteria (NFMC), Tiruchirapalli. ASN III medium at pH 7.5 was best suited for the growth of the alga. Axenic cultures were maintained at temperature of 26° ± 2°C. The experiments were carried out in 250 ml conical flasks, contained 100 ml of ASN III medium. The exponentially growing algal suspension was centrifuged and inoculated in the flasks containing different concentrations of NaCl such as 0.2, 0.4, 0.6 and 0.8M and ASN III medium grown cultures without NaCl were maintained as control upto 30 days.

The samples were drawn periodically at an interval of 10 days (10th, 20th and 30th day) for the analysis of biochemical parameters. The total chlorophyll contents were estimated by the method suggested by Arnon (1949), total proteins by Lowry *et al.* (1951). β-carotene accumulation according to Jensen (1978), total carbohydrates by Yemm and Willis (1954), Proline by Bates *et al.* (1973), and Glycine betaine was estimated by the method of Barak and Tuma (1981). All the experimental results were analysed and compared by ANOVA using SPSS package version 10.

RESULTS AND DISCUSSION

Oscillatoria treated with varying concentrations (0.2-0.8M) of NaCl revealed a slight increase in the total chlorophyll contents at lower concentrations of 0.2 and 0.4 M when compared to controlled set. It got decreased at higher

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concentrations of 0.6 and 0.8 M (Table 1). Reduced chlorophyll contents at higher salinities are due to decrease in photosynthetic rate because of salt osmotic and toxic ionic stress (Moradi and Ismail, 2007).

Exposure to different concentrations of NaCl, *Oscillatoria* exhibited decline in the total protein contents in all the concentrations of NaCl (Table 1). Similar observations were made by Sheikh *et al.* (2006) in *Anabaena cylindrica* when exposed to NaCl stress. However Hagemann *et al.* (1990) further reported, almost complete blockage of protein synthesis in cyanobacteria. β-carotene is an important source of provitamin-A is common amongst cyanobacterial strains exhibited an enhancement in all the concentrations of NaCl except 0.8 M (Table 1). Reddy *et al.* (2003) have observed similar results. Carbohydrate contents increased in all the concentrations of NaCl except 0.8 M upto 30th day (Table 2) Mackay *et al.* (1983). reported that, some cyanobacteria accumulate carbohydrates against osmotic stress. It was indicated that *Spirulina platensis* adapted to salinity stress by increasing carbohydrate metabolism in cells (Warr *et al.*, 1985; Vonshak *et al.*, 1988; Martel *et al.*, 1992). However, Gill *et al.* (2002) indicated that soluble sugars play an important role in the osmotic regulation of the cells during reproduction and stress conditions. The increase in sugar contents may be an adaptive measure under saline conditions.

The most distinctive change in the Proline accumulation which was increased in all the concentrations of NaCl grown cultures (Table 2). Kuznetsov and Shevyakova (1997), reported that proline increases the stress tolerance of the plants through osmoregulation and protection of enzymes against denaturation and the stabilization of protein synthesis. According to the observations of Imamul-Hug and Larther (1985), accumulation of proline may be considered as an indication of a salt induced water deficit. Fukutoku and Yamada (1984) placed on record that there is close connection between protein metabolism and proline accumulation.

It is interesting to note that glycine betaine content also increased in all the concentrations of NaCl except 0.8M upto 30th day (Table 2). Glycine betaine is the principal solute in highly salt tolerant halophilic forms. Reed *et al.* (1984) reported that, glycine betaine is characteristically found in most of the cyanobacteria under inhospitable conditions including *Aphanotheca halophytica* and *Synechocystis* DUN 52. Similar observations were made by Gabbay-Azaria *et al.* (1988) in *Spirulina subsala*. Record *et al.* (1988) reported that, osmolytes like glycine betaine and proline increase the cytoplasmic volume and free water content and permit cell proliferation under unfavorable conditions. However, Rhoades and Hanson (1993) reported that glycine betaine preserves thylokoid and plasma membrane integrity when exposed to saline solutions. Several species of marine algae have been reported to contain glycine betaine as a stabilizing osmolyte.

The study indicated that, *Oscillatloria willei* exhibited various response to different concentrations of NaCl. However the increased accumulation of biochemical contents at higher concentrations of NaCl may be an adaptive mechanism employed by *Oscillatoria* under NaCl stress. Hence, concerted efforts need to be under taken *in situ* to regulate physiological and biochemical processes under NaCl stress.

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Table 1 : Effect of Various Concentrations of NaCl on total Chlorophyll, total proteins and β-Carotene contents of *Oscillatoria willei*.

Cellular Metabolite	Con. of NaCl (M)	10th day	20th day	30th day
Total chlorophyll (µg/gFW)	Control	52.0 ± 0.03	65.0 ± 0.03	81.0 ± 0.02
	0.2 M	64.0 ± 0.04	79.0 ± 0.02	96.0 ± 0.03
	0.4 M	80.0 ± 0.02	95.0 ± 0.01	114.0 ± 0.03
	0.6 M	39.0 ± 0.01	48.0 ± 0.02	59.0 ± 0.04
	0.8 M	22.0 ± 0.03	35.0 ± 0.04	30.0 ± 0.01
Total Proteins (µg/gFW)	Control	88.0 ± 0.04	104.0 ± 0.02	121.0 ± 0.02
	0.2 M	69.0 ± 0.02	84.0 ± 0.08	93.0 ± 0.03
	0.4 M	52.0 ± 0.08	63.0 ± 0.02	72.0 ± 0.01
	0.6 M	42.0 ± 0.08	50.0 ± 0.08	37.0 ± 0.02
	0.8 M	22.0 ± 0.02	39.0 ± 0.02	20.0 ± 0.03
β-Carotene (µg/gFW)	Control	55.0 ± 0.04	73.0 ± 0.01	96.0 ± 0.04
	0.2 M	78.0 ± 0.04	95.0 ± 0.06	112.0 ± 0.02
	0.4 M	91.0 ± 0.04	116.0 ± 0.04	125.0 ± 0.03
	0.6 M	108.0 ± 0.02	128.0 ± 0.02	137.0 ± 0.02
	0.8 M	41.0 ± 0.04	62.0 ± 0.02	48.0 ± 0.04

Each value is expressed as mean ± S. D. (n=3) x Statistically significant at $P < 0.05$.
FW=Fresh Weight

Table 2 : Effect of Various Concentrations of NaCl on total Carbohydrates, Proline, and Glycine-betaine contents of *Oscillatoria willei*.

Cellular Metabolite	Con. of NaCl (M)	10th day	20th day	30th day
Total Carbohydrates (µg/gFW)	Control	45.0 ± 0.03	58.0 ± 0.01	69.0 ± 0.04
	0.2 M	61.0 ± 0.04	85.0 ± 0.02	99.0 ± 0.06
	0.4 M	77.0 ± 0.02	102.0 ± 0.05	112.0 ± 0.05
	0.6 M	90.0 ± 0.01	121.0 ± 0.03	135.0 ± 0.03
	0.8 M	30.0 ± 0.03	53.0 ± 0.04	65.0 ± 0.04
Proline (µg/gFW)	Control	38.0 ± 0.02	55.0 ± 0.01	73.0 ± 0.01
	0.2 M	63.0 ± 0.04	88.0 ± 0.02	108.0 ± 0.03
	0.4 M	87.0 ± 0.01	110.0 ± 0.01	127.0 ± 0.04
	0.6 M	107.0 ± 0.03	128.0 ± 0.02	140.0 ± 0.02
	0.8 M	121.0 ± 0.01	142.0 ± 0.01	153.0 ± 0.03
Glycine-betaine (µg/gFW)	Control	81.0 ± 0.02	95.0 ± 0.02	105.0 ± 0.01
	0.2 M	120.0 ± 0.01	131.0 ± 0.03	145.0 ± 0.02
	0.4 M	143.0 ± 0.02	154.0 ± 0.02	170.0 ± 0.01
	0.6 M	157.0 ± 0.02	172.0 ± 0.01	181.0 ± 0.03
	0.8 M	108.0 ± 0.01	120.0 ± 0.01	131.0 ± 0.01

Each value is expressed as mean ± S. D. (n=3) x Statistically significant at $P < 0.05$.
FW=Fresh Weight

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Comparative Evaluation of Growth of *Spirulina platensis* under Different Nutrient Media

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In the present investigation attempts were made to estimate and find out the growth in terms of chlorophyll content in different media *i.e.*, prescribed medium (CFTRI), petha waste medium and animal waste medium, under optimal laboratory culture conditions. The comparative study showed the best growth in prescribed medium followed by petha medium and animal waste medium. The chlorophyll content correlates with the growth of *Spirulina platensis*.

Keywords : *Spirulina,* Chlorophyll, Culture medium, Growth.

INTRODUCTION

The genus *Spirulina* has gained importance and international demand for its high value phytonutrients and pigments, which have applications in health foods, feed, therapeutics and diagnostics (Richmond, 1992; Becker, 1994). In recent years, *Spirulina* has become popular as a health food rather than just a source of protein. It represents the second most important commercial microalga for the production of biomass as health food and animal feed (after *Chlorella*) (Vonshak *et al.*, 2000). Among the various pigments produced by this organism, phycocyanin has attracted maximum attention. However, the self-renewing nature of the sources of chlorophyll has generated much commercial interest in recent years, due to its economic value. A major portion of commercial chlorophyll is used in the food industry, pharmaceutical and cosmetic industries (Kaushik *et al.*, 2009).

Spirulina is currently mass produced as a monoculture in outdoor cultivation systems (Venkataraman, 1995). Since this organism has a great capacity to grow in a wide range of media such as sewage water (Faucher, 1979), seawater (Saxena *et al.*, 1982), industrial effluents (Tanticharoen *et al.*, 1993) and human urine (Feng *et al.*, 2006) etc., many media have been developed along with standard medium-Zarrouk's medium. The aim of the present investigation was to evaluate the growth of *Spirulina platensis* in different nutrient media *i.e.* petha waste medium and animal waste medium under optimal laboratory culture conditions.

MATERIAL AND METHODS

Spirulina platensis is an alkalophilic organism exhibiting a growth rate of 0.113 $mgml^{-1}d^{-1}$, and protein content of 65%. This organism was obtained from the Centre for Conservation and Utilization of Blue Green Algae. IARI, New Delhi, and grown in 500ml flasks in CFTRI medium. Growth and maintenace of the culture was done at an illumination of 1600Lux light intensity at 30 ± 1°C under 12/12 h light-dark cycles. Manual shaking of cultures was done 3 - 4 times daily. The initial inoculum density was adjusted in terms of 0.025 or 0.00045 $mgml^{-1}$ dry weight or chlorophyll, respectively, in all the treatments.

CFTRI medium was used a control. Experiment was conducted in 500ml Erlenmeyer flask containing 250ml media in different ratios *i.e.*, 1:3, 1:1 and 3:1. Three sets of each medium *i.e.*, petha waste and animal waste were taken in triplicate. 10%of the prepared inoculums were added to the flasks. The flasks were covered perfectely by cotton plugs. Dry weight and chlorophyll content were determined on 28th day of inoculation. Dry weight determination was done by 20ml algal sample of suspension that was filtered through a Whatmann GF/C filter of 47mm diameter. The filtrate was dried in an oven for overnight at 70°C, put in desiccators for 20 min for cooling and weighted. Spectrophotometric estimation of the chlorophyll content was carried out following the method of Mackinney (Mackinney, 1941). Chlorophyll and protein values are expressed as $mgml^{-1}$ of culture suspension.

RESULTS AND DISCUSSION

A very famous dessert of Agra is patha, which is made by the pulp of petha (*Benincasa hispida*) fruit. The peel of fruit is thrown as waste which makes the surrounding unpleasant. To find the potentiality of this waste for growth of *Spirulina*, experiment was conducted and the results were compared with animal waste. The highest dry weight of *Spirulina* was found is Set III (3:1), having 25% modified medium [Table1,2]. The algal biomass obtained was 0.559 $mgml^{-1}$ and 0.525ml^{-1} in control, petha waste and animal waste respectively, which clearly shows the better yield in petha waste as compared to

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Table 1 : Growth efficiency of *Spirulina platensis* in control vis-a-vis petha waste medium.

Treatment	Dry Biomass ($mgml^{-1}$)	Chlorophyll ($x\ 10^{-3}\ mgml^{-1}$)
Control	0.559	10.31
Set I (3 : 1)	0.128	00.08
Set II (1 :1)	0.362	05.89
Set III (3 : 1)	**0.546**	**09.96**

animal waste. The growth of *Spirulina* was less in animal waste due to the high contamination. Several successful attempts have been made on the different media such as sewage water (Faucher, 1979) seawater (Saxena *et al.,* 1982), industrial effluents (Tanticharoen *et al.,*1993), human urine (Feng *et al.,* 2006) etc., to act as the source of nutrient media for *Spirulina* cultivation. Meanwhile, it helps in the environment cleanliness and act as ecofriendly.

Effluents from chicken and cow sheds and from piggeries were tested for growth of *Spirulina* by Yunus (1990) and found these effluents could be used as good substitutes for *Spirulina* cultivation. Pal and Chatterjee (1988) from little bicarbonate and EDTA as chelating agent supported good growth of *Spirulina platensis* for 10-15 days. Although the present investigation shows the less growth in petha waste than in CFTRI medium, but this indicate the potentiality of petha waste to act as nutrient medium for *Spirulina* cultivation. Further investigation is needed for further modification to improve its potentiality.

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Table 2 : Growth efficiency of *Spirulina platensis* in control vis-a-vis animal waste medium.

Treatment	Dry Biomass ($mgml^{-1}$)	Chlorophyll ($x\ 10^{-3}\ mgml^{-1}$)
Control	0.559	10.31
Set I (3 : 1)	0.120	0.002
Set II (1 :1)	0.345	04.24
Set III (3 : 1)	**0.525**	**07.87**

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Survey of Algal Diversity and its Quantitative Analysis in Godavari River at Nashik (M.S.), India

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Algal survey and its quantitative analysis at starting stretch of river Godavari was studied for two consecutive years (August 2003 to July 2005). The qualitative and quantitative analysis of algal forms was carried out during this period. Present study revealed that six algal groups were found during the investigation period *viz*, Chlorophyceae, Charophyceae, Dinophyceae, Euglenophyceae, Cyanophyceae and Bacillariophyceae. In all, 92 genera and 191 species of above classes are recorded during the study period, out of which Chlorophyceae comprised 105, Bacillariophyceae 38, Cyanophyceae 27, Euglenophyceae 16, Charophyceae 2, while Dinophyceae comprised 3 species. General spectrum of algal population showed that during monsoon months the algal population recorded was minimum, while during winter season its population was increased and during summer peak of development took place. Chlorophyceae was found to be a dominant group at all sampling stations. Water flow, water temperature, water level and alkalinity affect the algal population. At station S2 and S5 pollution tolerant genera were recorded indicating organic pollution of river water.

Keywords : Godavari river, Qualitative analysis, Phytoplankton.

INTRODUCTION

Algae are predominant organisms in water reservoirs and are primary producers of an ecosystem. The algal diversity, occurrence and composition of specific taxa in water is based on chemistry of water including gases and nutrients. On the basis of occurrence and diversity pattern algae were used as pullution indicator (Palmer, 1969; Rana and Palria, 1988; Nandan and Aher, 2005; Parvateesam and Mishra,1993). Hence, studying the diversity of algae pollution status of water body can be determined.

Godavari river has its origin from a small cave on the top of the hill called Brahmagiri in Sahyadri ranges of Western Ghats at Trymbakeshwar just 30 Kms upstream of Nashik city. It flows eastwards passing through Nashik city, Eklahare village, Darna Sangavi that is the point of confluence of Darna and Godavari rivers, and then passing through Saikheda comes to Nandur-Madhmeshwar dam or pick-up weir. Thus, passing through Niphad taluka of Nashik district, it enters in Kopargaon taluka of Ahmednagar district. Later on it enters in Gangakhed taluka of Aurangabad district and then passing through district Nanded, near Sironcha it enters in Andhra Pradesh and ultimately empties in Bay of Bengal.

Nashik district is situated on the great Deccan trap between 19° 35' and 20° 52' north latitude and 73° 16' and 74° 56' east longitude. It has an area of 15,582 sq.Km *i.e.*, 6015 square miles. Nashik city is situated at the starting stretch of Godavari river which releases huge quantities of domestic wastes and municipal sewage in river water which favours the luxurious growth of algal forms. The streams and rivers in India have been studied by many workers. However, there is no report on the algal diversity, its quantitative study and pollution status of water at starting stretch of Godavari river. Therefore, present study was undertaken to survey the algal diversity and evaluate the quantitative analysis of algae together with pollution status of water on the basis of pollution tolerant forms.

MATERIAL AND METHODS

As already mentioned, the Godavari river originates in Sahyadris of Western Ghats at Tryambakeshwar and passing through Nashik city, it flows east words. The present studies were carried out on the Godavari river for two years (August 2003 to July 2005). In all, five sampling stations were selected from Nashik city to Nandur-Madhmeshwar dam covering the stretch about 53 Kms Water samples were collected from following five study sites at monthly intervals.

Station S1:- This site is located 3 Km upstream of Ramkund at Panchawati.

Station S2:- This site is located at Ramkund at Panchawati, just 3 Km downstream of 1st site.

Station S3:- This site is located near the Eklahare village and 9 Kms downstream of Panchawati.

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Station S4:- This station is located at the confluence of Darna and Godavari rivers and 11 Kms downstream of station S3 near Darna-Sangavi village.

Station S5:- This site is located at south east of Nandur-Madhmeshwar pick up weir near the dam wall and just 30 Km downstream of station 4.

Monthly collection of water samples from above mentioned sampling sites were carried out between the period of 9.00 a.m. to 12.30 p.m. Surface water samples from the depth of 4 - 5 inches were collected in two liter sized acid cleaned plastic containers. For the estimation of dissolved oxygen (DO) and biological oxygen demand (BOD) the samples were collected in 250 ml sized glass stopper BOD bottles and fixed at the sampling sites. All the necessary observations were made at sampling sites. Water samples were analyzed for physical, chemical and biological (phytoplankton) parameters such as colour, light penetration, water temperature, electrical conductivity, total dissolved solids, pH, chloride, nitrate, phosphate, potassium, alkalinity, hardness, BOD, COD, free CO_2 dissolved oxygen and phytoplankton. Physico-chemical parameters were analysed by using the methods described in APHA (1985) and Trivedy and Goel (1986).

For algal study the water samples were collected by using plankton net of 25 meshes bolting silk. For qualitative study, samples were collected in separate bottles, while for quantitative analysis one liter water sample from each sampling site was collected and concentrated to 20 ml. All samples for the phytoplankton studies were preserved in 4% formaldehyde and Lugol's solution.

Algal forms were identified by using relevant literature like Desikachary (1959), Sarode and Kamat (1984), Prescott (1951, 54), Philipose (1967), Iyengar and Desikachary, (1981), Kaushik and Chauhan (2009), Anand (1998) and Bourrelly (1972) and enumerated. Algal forms were analyzed quantitatively by Lackey's drop method (Lackey, 1938).

RESULTS AND DISCUSSION

(a) Algal Composition : Monthly collection of algal forms was carried out during investigation period and analyzed quantitatively. Six algal groups were found during the investigation period *viz.* Chlorophyceae, Charophyceae, Dinophyceae, Euglenophyceae, Cyanophyceae and Bacillariophyceae. During present study, two genera of Charophyceae were collected in plankton net at certain stations and so they are also considered yet they are not planktonic algae. In all, 92 genera and 191 species of above classes are recorded during the study period. Out of these, Chlorophyceae comprised 105, Bacillariophyceae 38, Cyanophyceae 27, Euglenophyceae 16, and Charophyceae 2, while Dinophyceae consists of 3 species.

(b) Quantitative analysis of phytoplankton : Quantitative analysis of phytoplankton was carried out during the investigation period (Aug.2003-July2005). Monthly variations in quantitative abundance of phytoplankton at different sampling of Godavari river is depicted graphically (Fig.1-10).

General spectrum of planktonic algal population showed that during monsoon months the algal population recorded was minimum, during winter season its number was increased and during summer peak of development took place. In the first year of study period (August 2003-July 2004), during two months of monsoon (August 2003 and September 2003), monthly variations in quantitative abundance of phytoplankton showed maximum population. At station S2, in the months of August 2003 and September 2003 maximum population was found *i.e.,* 8.97 x 10^4 org./L and 13.13 x 10^4 org./L, respectively (Fig.3). Minimum population was recorded at station S1 *i.e.,* 3.21 x 10^4 org./L (Fig. 1).

In winter season (October 2003-January 2004), there was increase in population of phytoplankton. Maximum population was recorded during the month of January 2004 at station S3 *i.e.*,71.07 x 10^4 org/L (Fig.5) and minimum at station S4 *i.e.,* 7.51 x 10^4 org/L (Fig.8).

In first year of study period, maximum peak of phytoplankton abundance was noted during summer months (February to May). In April 2004, at station S3 maximum population was recorded (116.74 x 10^4 org/L), while in March 2003 minimum population was noted (11.6 x 10^4 org/L) at station S1 (Fig, 5,6).

In second year of study period, during monsoon the phytoplanktonic population recorded was very less. It could be due to flooding condition in rainy season as most of algae washed out. At station S2, maximum population was recorded during the month of September 2004 (17.96 x 10^4 org/L) and at station S1, minimum record was found (3.8 x 10^4 org./L). It is shown in Fig. 4 and Fig.1.

During the months of August 2004 and June 2005, there was no record of phytoplankton due to flooding condition.

During winter season (October 2004-January 2005) maximum algal population was recorded in the month of January 2005 *i.e.,* 79.6 x 10^4 org/L at station S2 (Fig.4) and minimum was found at station S1 (Fig.2) in October 2004 (4.4 x 10^4 org/L). Thus, during winter season there was increase in phytoplanktonic population. In second year also maximum peak development of phytoplanktonic abundance was found during summer (February 2004 to May 2005). In summer, maximum algal population was found at station S3 (113.82 x 10^4. org/L) (Fig 6) and minimum was recorded in March 2005 at station S1 *i.e.,* 9.13 x 10^4 org/L (Fig.2).

Likewise first year, minimum record of phytoplankton was noticed during monsoon season, which showed increase in number during winter, while during summer maximum peak was recorded. It was also observed that at stations S2 and S3 maximum algal population was found.

Maximum total phytoplanktonic yield was recorded at stations S2 (1273.18 x 10^4 org/L) followed by station S3 (996.4 x 10^4 org/L). Station S5 ranked third (736.66 x 10^4 org/L), while station S4 stood fourth (613.77 x 10^4 org/L) in total yield and station S1 showed minimum number of total phytoplanktonic

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population (213.64 x 10^4 org/L). Here point should be noted that at stations S1 water was flowing slowly and not much influenced by human activities, while at station S5 sometimes water remained in stagnant condition during investigation period.

Thus, during present study of Godavari river minimum phytoplanktonic population was found during monsoon season of both years. In the first year, (August 2003-July 2004) peak of algal development was in month of April 2004 at station S3 *i.e.*, 116.74 x 10^4 org/L (Fig. 5) and minimum phytoplanktonic population was noted during June 2004 *i.e.*, 1.3 x 10^4 org/L at station S1 (Fig. 1).

In the second year (August 2004-July 2005), maximum peak was recording during the month of April 2005 at station S3 *i.e.* 113.82 x 10^4 org/L (Fig. 6) and minimum population was noted at station S1 during the month of July 2005 *i.e.*, 1.1 x 10^4 org/L (Fig. 2). As water was flowing at this station it results the reduction in phytoplankton number.

Many workers studied the algae qualitatively and quantitatively. There are records of several investigators reporting that in temperate water, algae were found in maximum population during summer, while minimum in monsoon and winter seasons. Our results co-relates with Venkateswarlu (1969).

In India, Chakrabarty *et al.* (1959) reported summer maxima and winter minima of phytoplankton in the river Jamuna at Allahabad. Philipose (1959) recorded maximum development of algal population during summer and minimum during monsoon in the river Mahanadi. Our results matches with that of Laxminarayana (1965) who noted greatest crop of phytoplankton in winter and summer seasons and lowest number during monsoon from the river Ganges. According to him temperature and nitrogen maxima of summer were correlated with corresponding maxima of the phytoplankton abundance. Our results agreed with Pawar *et al.* (2006) who reported the maximum algal population during summer, medium during winter and minimum during monsoon season showing the dominance of Chlorophyceae.

Moore (1977) also reported the highest number of phytoplankton during summer season. Verma and Mohanty (1995) recorded more number of phytoplankton during summer. Our results co-relate with Ragothaman and Jaiswal (1995), who reported maximum population during winter and summer months and least in monsoon. Tiwari *et al.* (2001) also reported the maximum phytoplankton abundance during early summer and their number declined during monsoon from river Ganga.

Change in water level, transparency and temperature affected the growth and abundance of phytoplankton. Lakshminarayana (1965) was also of same opinion. Higher DO concentration was associated with winter maxima of phytoplankton. Thus, environmental factors were responsible for the phytoplankton productivity.

Pollution status : Algal community was used by many workers to monitor the water quality of various water reservoirs. Water pollution indices are based on this community, which are used commonly for detection and evaluation of water pollution.

According to Palmer (1969) the algal genera like *Euglena, Oscillatoria, Ankistrodesmus, Chlorella, Closterium, Scenedesmus, Cyclotella, Melosira, Gomphonema, Synedra, Phacus, Scenedesmus, Navicula* and *Nitzschia* are found in organically polluted water. Similar results were obtained during present study period at stations S2 and S3 indicating the organic pollution. Kanhere and Gunale (1999) reported the maximum values of nitrates and inorganic phosphates during summer, which favours the growth of pollution tolerant genera, which correlates with our results.

CONCLUSION

During present studies, in all, 92 genera and 191 species of algal taxa representing 16 orders and 34 families were recorded from all 5 sampling stations of the Godavari river. Maximum algal number was recorded during summer season, while during monsoon its number recorded was less. Quantitative analysis of phytoplankton showed maximum population at stations S2 and S3, while low population was recorded at station S1. Chlorophyceae was found to be a dominant group at all sampling stations during investigation period. Water flow, water temperature, water level and alkalinity affect the algal population. Pollution tolerant genera indicate the organic pollution at station S2 and S3.

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Graphical presentation of quantitative analysis of phytoplankton :

Fig. 1 : Monthly variation in quantitative abundance (no. of org./L x 10^4) of phytoplankton at sampling station S1 for the period August 2003 to July 2004.

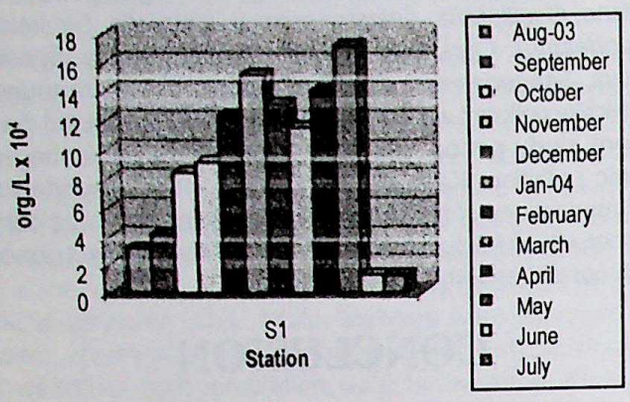

Fig. 2 : Monthly variation in quantitative abundance (no. of org./L x 10^4) of phytoplankton at sampling station S1 for the period August 2004 to July 2005.

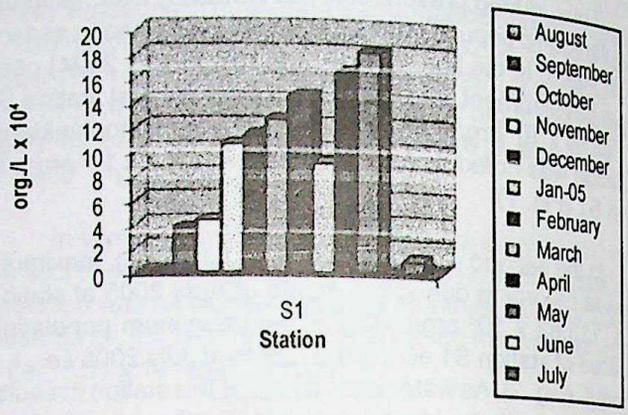

Fig. 3 : Monthly variation in quantitative abundance (no. of org./L x 10^4) of phytoplankton at sampling station S2 for the period August 2003 to July 2004.

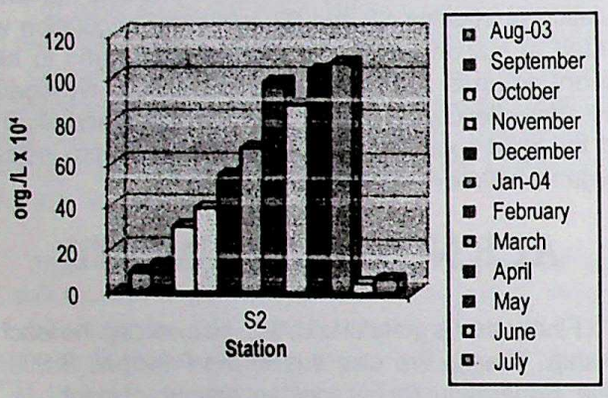

Fig. 4 : Monthly variation in quantitative abundance (no. of org./L x 10^4) of phytoplankton at sampling station S2 for the period August 2004 to July 2005.

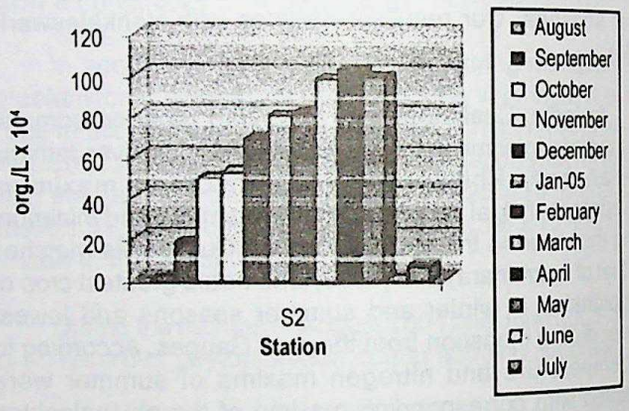

Fig. 5 : Monthly variation in quantitative abundance (no. of org./L x 10^4) of phytoplankton at sampling station S3 for the period August 2003 to July 2004.

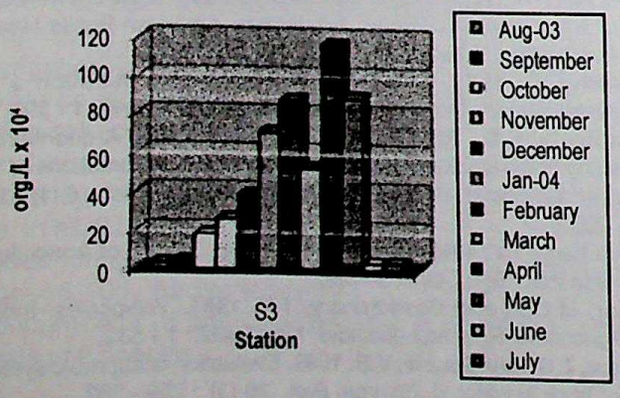

Fig. 6 : Monthly variation in quantitative abundance (no. of org./L x 10^4) of phytoplankton at sampling station S3 for the period August 2004 to July 2005.

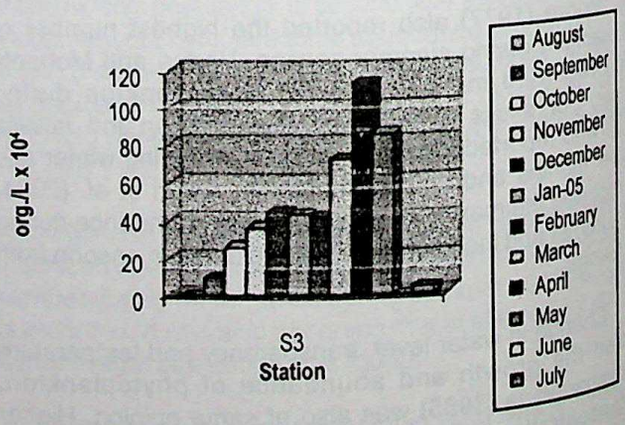

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Fig. 7 : Monthly variation in quantitative abundance (no. of org./L x 10^4) of phytoplankton at sampling station S4 for the period August 2003 to July 2004.

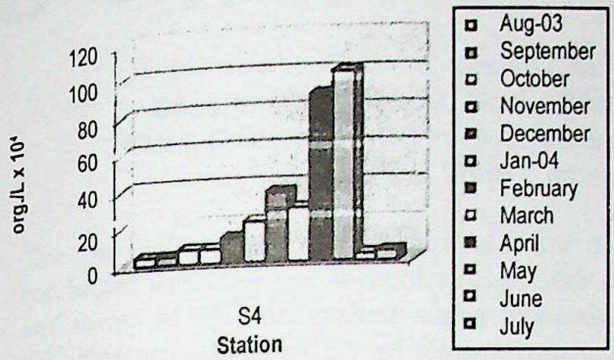


Fig. 8 : Monthly variation in quantitative abundance (no. of org./L x 10^4) of phytoplankton at sampling station S4 for the period August 2004 to July 2005.

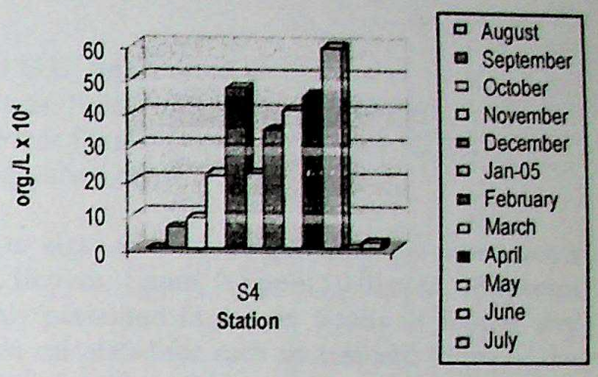


Fig. 9 : Monthly variation in quantitative abundance (no. of org./L x 10^4) of phytoplankton at sampling station S5 for the period August 2003 to July 2004.

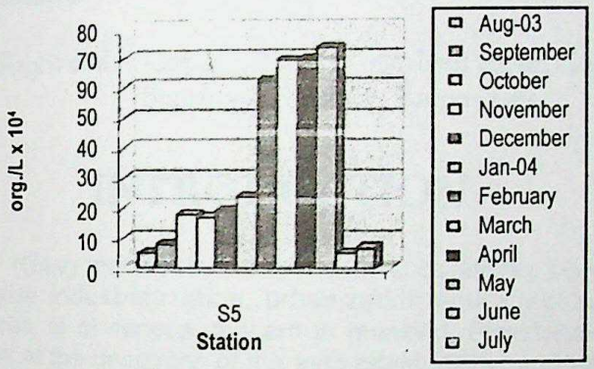
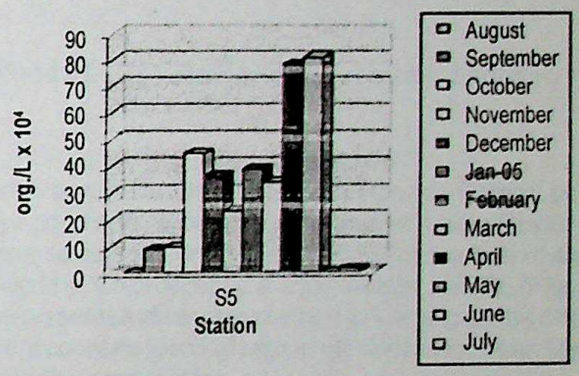


Fig. 10 : Monthly variation in quantitative abundance (no. of org./L x 10^4) of phytoplankton at sampling station S5 for the period August 2004 to July 2005.



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Responses of Chlorococcales Algae to Copper

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The toxic effect of copper on various forms of chlococcalean algae were investigated. In the experiments conducted with different concentrations of salt *viz*, 100 ppm, 10 ppm, 1 ppm, 0.1 ppm., 0.01ppm, 18 species and forms of chlorococcalean algae were almost uniformly presented in the six ponds of Raipur city (Chhattisgarh). Average No. of disappearance days had been calculated for each species and forms of the algae from the different ponds. With the help of these observations, comparative toxicity (high to low) at different concentrations of element with different algae have been estimated. Average number of days of survival of different species of chlorococcalean algae, with different concentrations of the copper taken for experiments presently exhibited that all the species of algae disappeared within 24 hours of exposure to 100 ppm of the salt, some of the forms of algae proved to be most sensitive and some were the most tolerant species.

Keywords : Copper, Chlorococcalean algae, Concentrations, Ponds, Disappearance days, Toxicity, Survival, Sensitive, Tolerant, Raipur city.

INTRODUCTION

Heavy metal pollution in aquatic ecosystems, through intensive industrialization, urbanization and agricultural practices, is of serious concern to mankind. Planktons are located at the beginning of the food chain (Jindal and Kaur, 2000). Heavy metal pollution can remove them resulting in complete destruction of the system. Heavy metals comprise an ill-defined group of metallic elements of density greater than 5. They have diverse physical, chemical and biological properties but generally all of them exert toxic effects on microorganisms. Algal communities have been used for monitoring the effect of industrial pollution (Cairns *et al,* 1978), nevertheless, algal community structure in a localized area is not caused by fortuitous circumstances or opportunistic behavior. The essential elements such as copper, zinc, cobalt have important biochemical functions in algae. The concentration of these trace elements is generally higher in the organisms than in the aquatic system and if it is otherwise, the metal content in the organism is regulated by homeostatic control mechanism (Bryan and Hummerstone 1973). At higher concentration of heavy metals the homeostatic mechanism get disrupted. Thus, during bioaccumulation of the heavy metals, the organism may be damaged (Venkataraman *et al*, 1992).

Ponds of Raipur city area are receiving huge amounts of sewage and sullage. Polluted water of these ponds are supporting several groups of algae. One of the important group, with respect to density, is chlorococcales. This group was selected for present studies. In the present studies, six ponds *viz*:, Ama pond, Budha pond, Dabri pond, Katora pond, Narsaiya pond and Telibandha pond located within Raipur city area were selected. Identification and Nomenclature of algae is followed by '*Chlorococcales*' by Philipose (1967), (Figs. 1-18). The present studies were planned to investigate the effects of different concentrations of copper on chlorococcalean algae, present in the pond water.

MATERIAL AND METHODS

Samples were collected in plastic cans. Before collecting the sample, the cans were washed throughly. Surface water only was collected from the ponds, for experimentation. Glass jars of four liter capacity were filled with 3 liters of pond water was taken in each of the jar. To this water, standard solution of copper was added to make the concentration of the solution to 100 ppm, 10 ppm, 1 ppm, 0.1 ppm and 0.01 ppm respectively. To experiment with the water from different ponds a set of six jars was prepared for each pond. One of the jars was maintained as control. Remaining five jars were prepared, one each with 100 ppm, 10 ppm, 1 ppm, 0.1 ppm and 0.01 ppm of the copper. The glass jars were fitted with air bubbles. Bubbling was done at the lowest speed. This was done to keep the algae floating, because neither, keeping the sample in jars resulted in settling of the algae. Before applying the heavy metal solution, 5 ml of water was taken to observe it as control. Thereafter, 5 ml water sample was taken from each of the jars, on alternate days. This alternate day collection of sample was continued till complete disappearance of algae in the sample. Density was recorded with a gap of 1 or 2 days. Change in density was followed till the days of complete

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disappearance of all the species. Change in density was recorded separately for all the species and all the pond water selected for studies. Reaction, in the form of survival or death, of the chlorococcalean algae, towards presently taken concentrations (100, 10, 1, 0.1 and 0.01 ppm) of metal had always been exhibited in the form of death, sooner or later of all the chlorococcalean forms. Death has been recorded as complete disappearance of the algae from the experimental jars. Disappearance of the algae for each of the ponds was computed concentration wise. Average No. of disappearance days had been calculated for similar concentration *viz:* 100 ppm, 10 ppm, 1 ppm, 0.1 ppm and 0.01 ppm separately each species and form of the algae from the different ponds. With the help of these observations, comparative toxicity (high to low) at different concentrations of element with different algae have been estimated. In all the cases, pond water, in which the algae were present, had been taken as the medium for the experiment. Variation in physico-chemical characters between pond waters can not be ruled out. However, considering the much drastic effects of the experimental elements, taken presently within the concentration range of 0.01 ppm to 100 ppm, the effect of differences, in physico-chemical characters of pond water has been taken to be non-significant.

RESULTS

Variation in density of chlorococcalean algae on successive days after the addition of different concentrations of copper in the water of different ponds.

With 100 ppm concentration, all the species and forms died before first observation date after the addition of salt.

AMA POND: In the water sample, set for experimentation with copper *Scenedesmus bijugatus* and *Scenedesmus armatus* var. *bicaudatus* were the most abundant species with *Selenastrum minutum* as the least abundant species. Copper exhibited much more drastic effect. Decrease in density after the addition of different concentrations of copper was very steep on successive days. Out of 11 species, 7 were able to survive up to 2 days with 10 ppm concentration of copper but none of the species could survive up to 4 days with this much concentration of copper. Concentration was related also to the speed of the decrease in density being more steep with higher concentration and gentle with lesser concentration.

BUDHA POND: In the water sample, taken for experiments with copper *Chlorococcum infusionum* was the most dense species followed closely in density by *Scenedesmus quadricauda* and *Chlorella vulgaris* upto 2 days after the addition of copper, up to 2 days density was very little affected with 0.01 ppm concentration of copper in the water. However, with higher concentration and from 4 days onwards after the addition of copper steeply declining trend in density was observed.

DABRI POND: *Scenedesmus bijugatus, Scenedesmus armatus* var. *bicaudatus* and *Scenedesmus quadricauda* all three had far higher density as compared to other 5 forms, in the initial sample. None of the forms could survive up to 3 days with 10 ppm copper but with significant difference that none of the forms was able to survive beyond 7 days with any of the concentration of copper. Further, there were only 3 forms which could be recorded on 7th day after the exposure with even 0.01 ppm concentration of copper.

KATORA POND: *Scenedesmus bijugatus* with 117.84 x 10^3 individuals/ml was the most dominant species followed closely by *Scenedesmus armatus* var. *bicaudatus* with 108 x 10^3 individuals/ml. After the addition of copper to the water at 10 to 0.01 ppm concentrations, the density of algae decreased at all the concentrations. However, the decrease in absolute density was much related to the initial density with less dense species lesser in density and more dense species with higher density on different days after the addition of copper.

NARSAIYA POND: *Scenedesmus bijugatus* and *Scenedesmus armatus* var. *bicaudatus* were most dominant species density wise with almost equal density. Decrease in density was more sudden after 4 days after the addition of copper. However, survival period of different species was related to their initial density *Micractinium pusillum* with lowest density of 14.72 x 10^3 individuals/ml was first to disappear after 4 days, while species with higher initial density species could survive up to 7 days, at least with lowest concentration of copper.

TELIBANDHA POND: The initial density of algae in the water taken for experiment, *Scenedesmus bijugatus, Scenedesmus armatus* var. *bicaudatus* and *Scenedesmus quadricauda* had far higher density than the other five species. The species with higher density maintained their higher density also on different days after the addition of copper. The effect of copper was more drastic on the algae as none of the form was able to survive beyond 6 days with any of the presently added concentration (10 ppm to 0.01 ppm) of copper.

Average number of days of survival of different species of chlorococcalean algae, with different concentrations of the element taken for experiments presently exhibited (Table & Fig.) that all the species of algae disappeared within 24 hours of exposure to 100 ppm of the salt. The concentration of 10 ppm of copper in the water, killed *Scenedesmus dimorphus* and *Chlorella vulgaris* as was observed with 100 ppm concentration of copper with 10 ppm of copper, out of 18 chlorococcalean forms recorded in the samples as many as 13 of the forms could survive at the maximum only for 2 days. With 1 ppm concentration, *Scenedesmus dimorphus* was most sensitive and maximum increase in survival time of 4.33 days was observed with *Scenedesmus quadricauda*, As many as 8 of the species exhibited average survival of 4 days with 1 ppm concentration of copper. In 0.1 ppm of cobalt solution survival to the maximum with 6 days was observed with *Scenedesmus armatus* var. major, *Scenedesmus abundans* and *Micractinium pusillum*. *Scenedesmus dimorphus* exhibited survival period of only 2 days (minimum) with 0.1 ppm of copper. Lowest concentration of copper, presently experimented with, was 0.01 ppm. Lowest survival period was only 2 days recorded for *Scenedesmus dimorphus*. *Schroederia indica* proved to be the most resistant species at 0.01 ppm concentration of copper.

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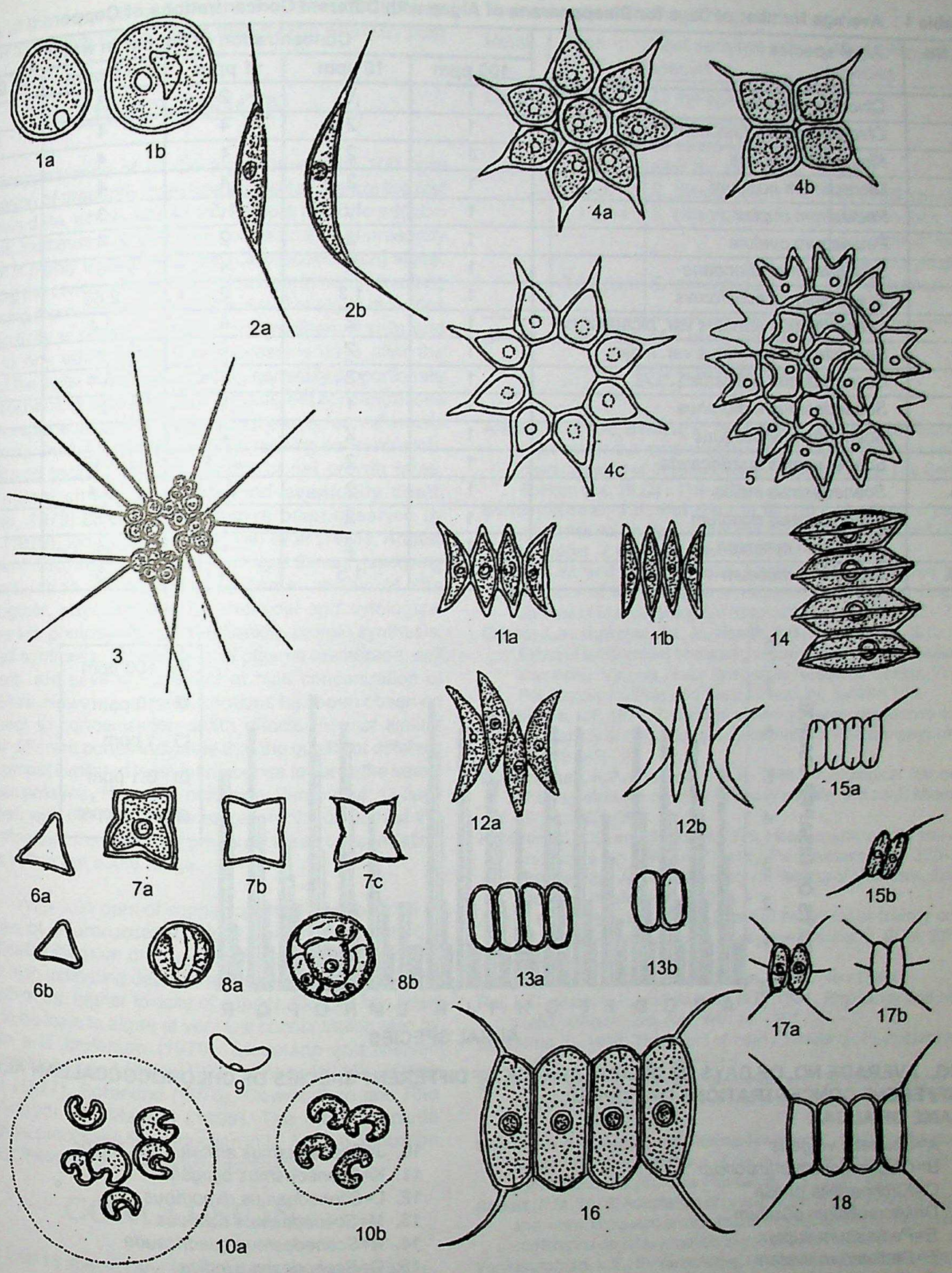

Figs. 1-18. 1a-b, *Chlorococcum infusionum*; 2a-b, *Schroederia indica*; 3, *Micractinium pusillum*; 4a-c, *Pediastrum ovatum*; 5, *Pediastrum duplex*; 6a-b, *Tetraedron muticum*; 7a-c, *Tetraedron minimum*; 8a-b, *Chlorella vulgaris;* 9, *Selenastrum minutum*; 10a-b, *Kirchneriella obsea*; 11a-b, *Scenedesmus obliquus*; 12a-b, *Scenedesmus dimorphus*; 13a-b, *Scenedesmus bijugatus*; 14, *Scenedesmus acutiformis*; 15a-b, *Scenedesmus armtus* var. *bicaudatus*; 16, *Scenddesmus armatus* var. major; 17a-b, *Scenedesmus abundans*: 18, *Scenedesmus quadricauda*.

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Table 1 : Average Number of Days for Disappearane of Algae with Different Concentrations of Copper.

S.No.	Algal species	Concentration of copper in water				
		100 ppm	10 ppm	1 ppm	0.1 ppm	0.01 ppm
1.	*Chlorella vulgaris*	1	1	2	3	5
2.	*Chlorococcum infusionum*	1	2	4	4	5.75
3.	*Kirchneriella obesa*	1	2	4	4	6
4.	*Micractinium pusillum*	1	2	4	4	6
5.	*Pediastrum duplex*	1	1.5	1.5	3	4
6.	*Pediastrum ovatum*	1	2	2	4	6
7.	*Scenedesmus abundans*	1	2	4	6	6
8.	*Scenedesmus acutiformis*	1	1.3	2	2.66	4
9.	*Scenedesmus armatus* var. *bicaudatus*	1	2	4	5.4	6.6
10.	*Scenedesmus armatus* var. major	1	2	4	6	6
11.	*Scenedesmus bijugatus*	1	2	4	5.4	6.6
12.	*Scenedesmus dimorphus*	1	1	1	2	2
13.	*Scenedesmus obliquus*	1	2	2	3	4
14.	*Scenedesmus quadricauda*	1	2	4.33	5.66	6.16
15.	*Scenedesmus indica*	1	2	3.2	5.4	6.8
16.	*Scenedesmus minutum*	1	2	2.66	3.33	4
17.	*Tetraedron minimum*	1	2	4	4	6
18.	*Tetraedron muticum*	1	1.5	4	5	5

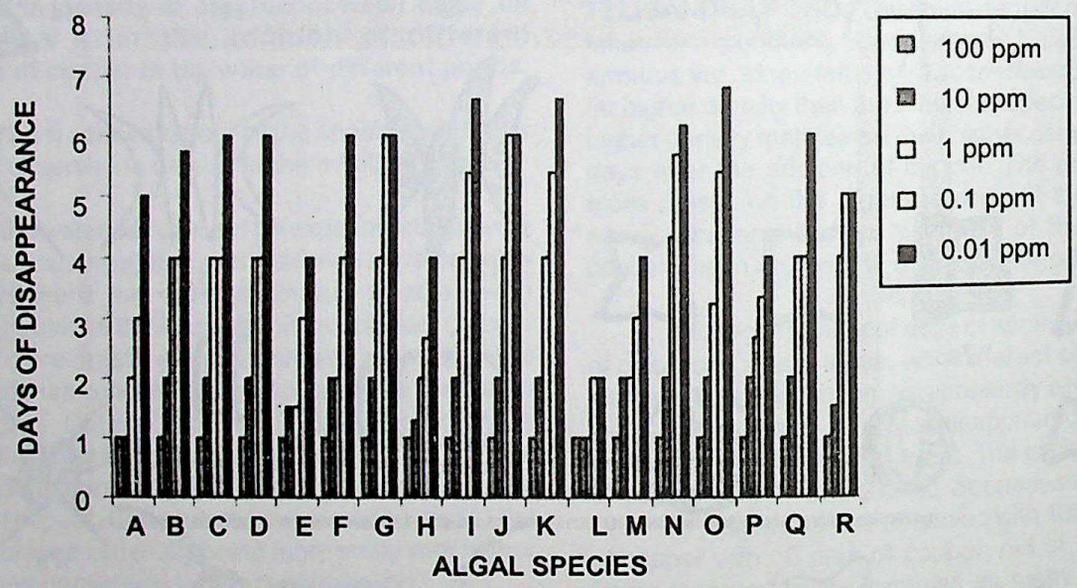

FIG.: AVERAGE NO. OF DAYS OF DISAPPEARANCE OF DIFFERENT SPECIES OF CHLOROCOCCALEAN ALGAE WITH DIFFERENT CONCENTRATIONS OF COPPER.

NAME OF ALGAE

1. A=*Chlorella vulgaris*
2. B=*Chlorococcum infusionum*
3. C=*Kirchneriella obesa*
4. D=*Micractinium pusillum*
5. E=*Pediastrum duplex*
6. F=*Pediastrum ovatum*
7. G=*Scenedesmus abundans*
8. H=*Scenedesmus acutiformis*
9. I=*Scenedesmus armatus* var. *bicaudatus*
10. J=*Scenedesmus armatus* var. major
11. K=*Scenedesmus bijugatus*
12. L=*Scenedesmus dimorphus*
13. M=*Scenedesmus obliquus*
14. N=*Scenedesmus quadricauda*
15. O=*Scenedesmus indica*
16. P=*Scenedesmus minutum*
17. Q=*Tetraedron minimum*
18. R=*Tetraedron muticum*

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surviving at the average for 6.8 days. 9 of the total 18 forms were able to survive for 6 to more than 6 days with 0.01 ppm of copper.

DISCUSSION

Disappearance of all the algal forms, with 100 ppm concentration of presently investigated the salt, before the first observation date, which was 48 to 72 hours after the addition of the salt, indicates that 100 ppm concentration of presently taken salt is highly toxic to all of the (Chlorococcalean) algae. Decreasing the concentration of the elements in water, resulting in increasing the number of days of survival of algae indicates that the toxicity is only due to the concentration of salts and not due to any other factor. On successive days after the addition of the salts, the algae exhibiting generally proportionate decrease is because the effects of the different concentrations of the elements is almost similar for all the species. All metals exhibit similar inhibitory effects with increasing concentration. These effects include the depression of net growth rates, morphological changes in cells and eventually death (Sorentino, 1978). Similar results have been observed by Whitton, (1970), De Filippis (1979), Rai *et al* (1981), Angadi and Mathat (1998) that heavy metal toxicity is through poisoning and inactivation of enzyme systems, many of the morphological, physiological, biochemical and cytological processes *viz*: photosynthesis, respiration, protein synthesis, chlorophyll synthesis, permeability of plasma membrane, cell division etc. are severely affected at high concentration of heavy metals. However, some exceptions had been observed with respect to concentration-death effects. Almost similar results for different ponds indicates that the quality of different ponds is almost similar at least for response towards the heavy metal taken presently for experimentation. Survival for, 11 days at the most, with the lowest concentration of 0.01 ppm of the salt indicates that the effects of presently taken concentration of salts is within the acute range.

With 0.01 ppm of copper, survival of all but one of the species of chlorococcalean algae, ranging from 4 to 6.8 days, indicates its more drastic effects. Lesser concentration of copper also indicating death of algae within shorter interval again confirm the higher toxicity of copper. Copper has been reported to be toxic to algae at very low concentration also by Steemann and Anderson (1970), Steemann and Nielson (1970), Hutchinson and Stoks (1975), Stokes (1975), Bentley and Reid (1977), Sorentino (1978), Gowrinathan and Rao (1989), Angadi and Mathad (1998). The effects include physiological processes like reduction in N_2 fixation, reduction in photosynthesis and chlorophyll degradation.

CONCLUSION

In total 18 species and forms of chlorococcalean algae, recorded in the water sample from the different ponds, set up for experiments with different concentration of metal, some of the forms of algae proved to be most sensitive and some were the most tolerant species.

These are shown in the table given below:-

Metal	Conc.	Most sensitive species	Most tolerant species
Copper	10 ppm	All the species disappeared within 24 hours to 48 hours after the exposure.	---
	1 ppm	*S. dimorphus*	*S. quadricauda*
	0.1 ppm	*S. dimorphus*	*S. abundans, S. armatus* var. major, *M. pusillum.*
	0.01 ppm	*S. dimorphus*	*Schroederia indica*

S = Scenedesmus, M = Micractinium,

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Enumerated Cyanobacterial Flora of Rice Grown Soil of Agra District

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Present studies enumerate 26 species of Cyanobacteria belonging to family-Cyanophyceae isolated from soil of different selected sites at Agra. These strains were culture *in vitro* and utilized as biofertilizer for conditioning the saline soil. Present investigation has been made to observe the Cyanobacterial species present in Agra soil.

Keywords : Cyanobacteria, Algal flora of soil, BGA and Rice crop.

INTRODUCTION

Agra city is one of the major tourist place in India. The region has got unique assemblage of Cyanobacterial flora and fauna constituents of Agra soil. Cyanobacteria belong to family Cyanophyceae and are present in all irrigated agricultural soil including crops like Rice, Wheat, Barley and Pea. (Singh, 1961; Venkataraman, 1972; Kanniayan, 2000) observed that Cyanobacteria play an important role in organic farming. Potts (2000) worked on predominance of *Nostoc* spp. in terrestrial ecosystem and its contribution to nitrogen enrichment of nutrient poor soil. There are few reports on algal elements of Eastern Himalayas by-Dickie (1987). Suseela and Toppo (2006) were also observed systematically 45 members of Cyanophyceae, 14 members of Chlorophyceae,

Enumeration of Cyanobacterial taxa:

S.No.	Cyanobacterial flora	Cells in diameter	Locality	Habitat
1.	*Anabaena oryzae*	2.5 - 3 µm	On the surface of moist soil.	
2.	*A. torulosa*	5 µm	Free floating in irrigated water.	
3.	*A. utermohli*	6 µm	Wet land of roadside of the field.	↑
4.	*A. variabilis*	4 µm	On the surface of moist soil.	
5.	*Aphanocapsa banaresensis*	12 µm	On the surface of moist soil.	
6.	*Aphanothece castagnei*	9 µm	In water logged soil.	
7.	*Aulosira pseudoromo*	8 µm	Free floating in irrigated water.	
8.	*Calothrix* spp.	5 - 8 µm	Associated with mosses in canal.	
9.	*Chroococcus minutus*	4 - 10 µm	Planktonic in a water canal.	
10.	*Cylindrospermum sphaerica*	4 µm	On the surface of wetland.	
11.	*Gloeocapsa granosa*	8 µm	In water logged soil.	
12.	*G. montana*	15 - 25 µm	On the surface of water canal.	FRESH
13.	*G. rupestris*	6 µm	Associated with water algae.	
14.	*Lyngbya birgei*	18 µm	Free floating in water.	WATER
15.	*Microcystis aeruginosa*	3 - 4.2 µm	Planktonic in a water canal.	
16.	*Nostoc commune*	4 - 5 µm	On the surface of wetland.	AND
17.	*N. comminutum*	3.7 - 4 µm	On the sides of water canal.	
18.	*N. punctiforme*	3 - 6 µm	On the surface of water canal.	SOIL
19.	*Oscillatoria chlorina*	5 - 8 µm	Associated with mosses in canal.	
20.	*O. formosa*	6 µm	On the surface of moist soil.	
21.	*O. granulata*	5 µm	On the surface of moist soil.	
22.	*O. irrigua*	4 - 8 µm	Free floating in irrigated water.	
23.	*O. limosa*	17 µm	Free floating in water.	
24.	*O. tenuis*	8 µm	Free floating in water.	
25.	*O. quadripunctulata*	8 µm	On the surface of wetland.	↓
26.	*Phormidium retzii*	8 µm	On the surface of water canal.	

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Table 1 : Distributional pattern of Cyanobacterial flora in rice soil of Agra.

S.No.	Cyanobacterial flora	Selected sites of Agra district			
		Dayalbagh	Keetham	Shrinagar (Tundala)	Etmadpur
1.	*Anabaena oryzae*	+	+	-	-
2.	*A. torulosa*	+	+	-	+
3.	*A. utermohli*	-	-	+	-
4.	*A. variabilis*	+	-	-	+
5.	*Aphanocapsa banaresensis*	-	-	+	-
6.	*Aphanothece castagnei*	-	-	+	-
7.	*Aulosira pseudoromo*	+	-	+	+
8.	*Calothrix* spp.	-	+	-	+
9.	*Chroococcus minutus*	-	+	-	-
10.	*Cylindrospermum sphaerica*	-	+	-	+
11.	*Gloeocapsa granosa*	+	+	+	-
12.	*G. montana*	+	+	+	-
13.	*G. rupestris*	-	+	-	-
14.	*Lyngbya birgei*	-	+	-	+
15.	*Microcystis aeruginosa*	+	+	-	-
16.	*Nostoc commune*	+	-	-	-
17.	*N. comminutum*	-	-	+	-
18.	*N. punctiforme*	+	+	-	+
19.	*Oscillatoria chlorina*	-	-	+	-
20.	*O. formosa*	+	+	-	-
21.	*O. granulata*	+	+	+	+
22.	*O. irrigua*	+	+	-	-
23.	*O. limosa*	+	-	-	+
24.	*O. tenuis*	+	-	-	-
25.	*O. quadripunctulata*	-	-	-	+
26.	*Phormidium retzii*	+	-	-	+

8-Bacillariophyceae and one-Xanthophyceae under the study of algal flora of Gangtok, Sikkim.

Cyanobacterial inoculation was found to have a significant additive effect at all levels of nitrogen fertilizers applied in the form of ammonium sulphate (Aiyer *et al.*, 1972). Some evidences indicated that the part of the nitrogen requirement for the rice crop (25-35%) could be met by using Cyanobacteria as biofertilizer (Ghose and Saha, 1997; Rai *et al.*, 2000).

MATERIAL AND METHODS

Soil samples were collected from 4 different sites of Agra *viz.*, Dayalbagh, Keetham, Shri Nagar and Etmadpur in August-2006. The Cyanobacterial cultures were isolated from soil and culture on BG-11 medium (Stainer *et al.*, 1971) at 30 ± 2°C at 2000-2500 lux light *in vitro*. Different algal strains were examined under the research microscope and the camera lucida diagrams were drawn. Species identification was based on the keys given by Desikacharya (1959).

RESULTS AND DISCUSSION

The distribution pattern of Cyanobacteria was studied in four sites of Agra district by enrichment culture technique and in all the samples, *i.e.*, 26 forms of Cyanobacteria were recorded. The majority of the forms were filamentous in nature. The unicellular / colonial genera were found in all the sites of Agra. The filamentous non-heterocystous forms were represented by *Phormidium* sp., *Plectonema* sp. and *Oscillatoria* sp. Whereas the heterocystous nitrogen fixed forms were the species of *Anabaena, Calothrix, Cylindrospermum, Nostoc* and *Aulosira* sp.

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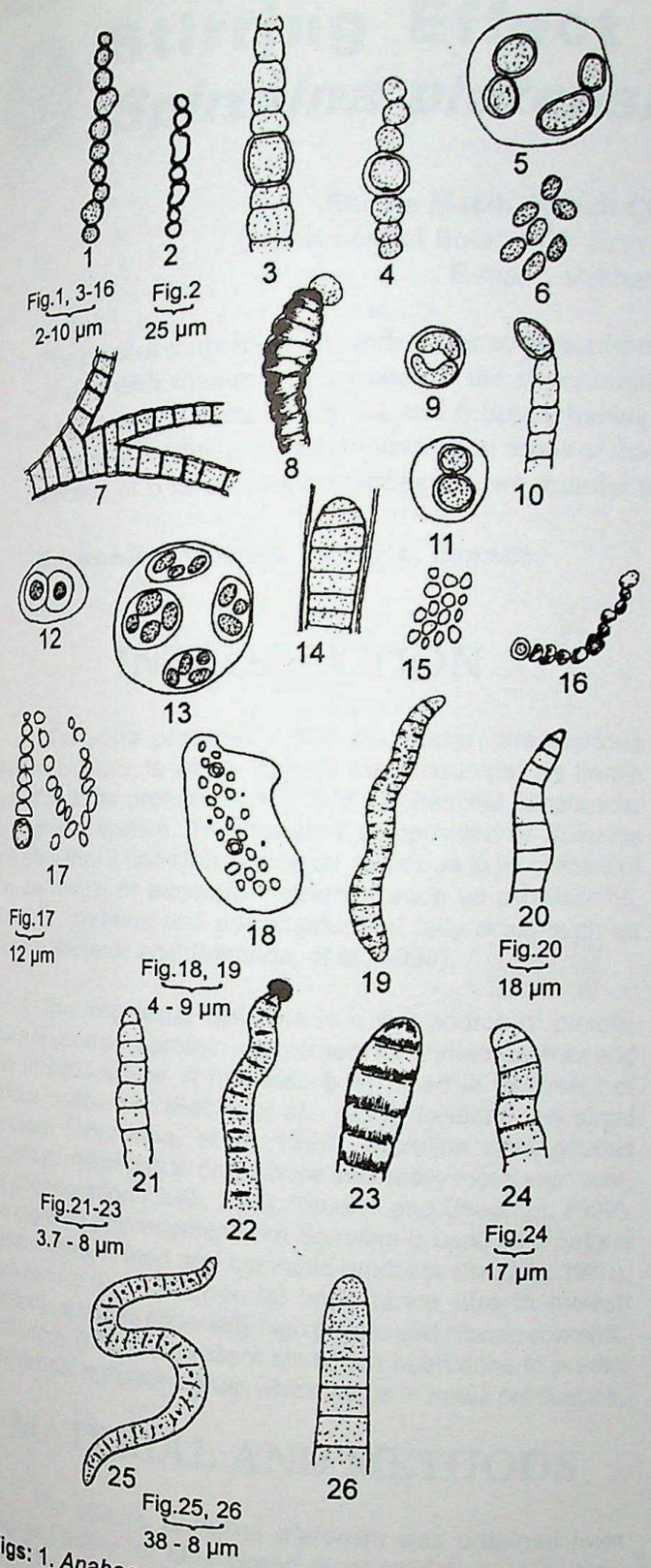

Figs: 1. *Anabaena oryzae* 2. *A. torulosa* 3. *A. utermohili* 4. *A. variabilis* 5. *Aphanocapsa banaresensis* 6. *Aphanothece castagnei* 7. *Aulosira pseudoromo* 8. *Calothrix* sps. 9. *Chroococcus minutus* 10. *Cylindrospremum sphaerica* 11. *Gloeocapsa granosa* 12. *G. montana* 13. *G. rupestris* 14. *Lyngbya birgei* 15. *Microcystis aeruginosa* 16. *Nostoc commune* 17. *N. comminutum* 18. *N. punctiforme* 19. *Oscillatoria chlorina* 20. *O. formosa* 21. *O. granulata* 22. *O. irrigua* 23. *O. limosa* 24. *O. tenuis* 25. *O. quadripunctulata* 26. *Phormidium retzii*.

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Stirring Effect on the Production of *Spirulina platensis*

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The present study has been undertaken to prescribe the optimal stirring conditions for the successful production of alga with maximum biomass. In the experiment nine flasks (1L) in three sets were agitated for 2,4 and 6 minutes at an interval of 3,4 and 6 hours, having comparative study with the fourth set kept undisturbed (control). Productivity was measured in terms of dried biomass (g/l). It was observed that two times stir at an interval of 6 hours in a single day for two minutes to be the best with regard to the biomass production.

Keywords : *Spirulina,* Agitation, Biomass.

INTRODUCTION

Spirulina platensis, a multicellular, filamentous cyanobacterium, is widely used in many countries as health food due to its protein content and biochemical substances for immune system. The chemical composition of *Spirulina* indicates that it has high nutritional value due to its content of a wide range of essential nutrients, such as provitamins, minerals, proteins and polyunsaturated fatty acids such as gamma linolenic acid (Miranda, *et al.,* 1998).

The microalga, *Spirulina* is a rich source of protein, which is used as a protein supplement for humans, chicks and also in aquaculture. It has also been used in treatment of various anticancer (Babu, *et al.*, 1995), lowering the blood pressure (Anuradha, *et al.*, 1999). *Spirulina* also reduces hepatic damage due to drug abuse and heavy metal exposure, cell degeneration (Bulik, 1993, Kaushik and Chauhan, 2009). The pigment phycocyanin from *Spirulina* is used as a natural colour in health food and cosmetic products (Switzer, 1980). *S.platensis* has commercial importance due to overall nutritional qualities especially high protein and vitamin contents, particularly B12. The present study has been done to predict out the best agitation value, which helps in mass production.

MATERIAL AND METHODS

The culture *Spirulina platensis* was obtained from National Facility for Blue Green Algal collection, IARI, New Delhi. The whole experiment was carried out for the period of one month *i.e.*, February 2009-March 2009, in the flasks of 1L. capacity, such flasks were filled with 400 ml of freshly prepared CFTRI medium and each flask was inoculated with 100ml of mother culture. There were four sets of experimentation:

1. Nine flasks in 3 sets of three each were agitated for 2,4 & 6 minutes at the time interval of 3 hours from 10 O'clock & 4 O'clock respectively.
2. Next nine flasks in 3 sets of three each were agitated for 2,4 & 6 minutes at the time interval of 4 hours from 8 O'clock, 12 O'clock & 4 O'clock respectively.
3. Other nine flasks in 3 sets of three each were agitated for 2,4 & 6 minutes at the time interval of 6 hours from 10 O'clock & 4 O'clock respectively.
4. Last nine flasks were kept undisturbed during the whole experimentation. This set was considered as control set. All four sets were subjected to same growth conditions such as temperature (20-28°C) light (800 lux -1800 lux) photoperiod 12h/d.

Harvesting process was carried out after 28 days with ordinary sieve to remove foreign particles. Finally it was filtered with the help of fine sieve of 500 µm. Slurry was washed 3 - 4 times with sterilized distilled water to remove the impurities. After washing, slurry was sun dried for 2 - 3 days.

RESULTS AND DISCUSSION

The effect of the agitation on *Spirulina* cultures were studied by providing different agitation conditions. The results are as follows:-

Set I- In first set of experiment, mean dried biomass were 0.545 g/l, 0.0562 g/l and 0.578 g/l, when the cultures were agitated for 2, 4 & 6 minutes at an interval of 3 hours respectively. (Table-1)

Set II- In second set of experiment, mean dried biomass were 0.546 g/l, 0.532 g/l and 0.529 g/l, when the cultures were agitated for 2, 4 & 6 minutes at an interval of 4 hours respectively. (Table-1)

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Table 1 : Mean dried biomass g/l of *Spirulina platensis* cultures agitated for different duration.

Time interval of Agitation (Hrs.)	Duration (Minutes)	Dried biomass (g/l)
3	2	0.545
	4	0.562
	6	0.578
4	2	0.546
	4	0.532
	6	0.529
6	2	0.619
	4	0.610
	6	0.591
Control	Nil	0.305

Set III- In third set of experiment, mean dried biomass were 0.619 g/l, 0.610 g/l and 0.591 g/l, when the cultures were agitated for 2, 4 & 6 minutes at an interval of 6 hours respectively. The best growth was found when the culture was stir for two times in a single day. (Table-1)

Set IV- In the fourth set, biomass production was found to be minimum. In this set the flasks were kept undisturbed, the mean dried biomass was 0.305 g/l. (Table-1)

Spirulina is a filamentous non-heterocystous alga which belongs to cyanobacteria in which sexual reproduction is absent *i.e.*, alga can multiply only by means of fragmentation and fission (Tomaselli, 1993). Single filament of alga is called trichome and is of two types straight and spiral. Straight trichomes are abundantly found when it is cultured on mass scale in comparison to spiral trichomes. In the process of fragmentation trichomes break down into daughter trichomes. These daughter trichomes are called harmogonia and develop into a new filament. In the present experiment, when the cultures were subjected to specific period of agitation (2 minutes twice a day) enhancement in biomass was observed. Beyond this specific period of agitation a decrease in the productivity was observed. The positive effect of agitation is the through mixing of nutrients in the culture medium so that alga receives their full nutrition resulting in high biomass as compared which were not agitated. Agitation creates not only turbulence in the culture but also helps the trichomes to move inside the culture media, which facilities each trichome to get sunlight.

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Effects of Cd^{2+} and Cu^{2+} on the Seedling Characteristics of *Pisum sativum* L.

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Influence of borderline metals like Copper and Cadmium had been well documented by some investigations (Whoolhouse, 1983; Gora *et al.*, 1985; Macquot *et al.*, 1986). The adverse influence of Copper in beans was recorded by Maksymiec abd Baszynski (1996). Copper induced enhancement of enzymatic activity in *Lactuca salva* was reported by Mukherji and Dasgupta (1972). Toxic effects of Cadmium had been established during itai-itai disease in Japan (Kobayashi, 1978). The effects of both Copper and Cadmium on seedling morpho-physiological parameters of *Pisum sativum* L. var B 22 have been studied in the present scope of investigation.

Cupric acetate and Cabmium sulphate were used as test chemicals. The concentration series of Copper was 0.1, 1.0, 10.0, 20.0, 30.0, 50.0, 100.0, 200.0 and 500.0 mg/l an that of Cadmium was 0.1, 1.0, 10.0, 20.0, 30.0 and 50.0 mg/l.

For germination studies, healthy seeds were selected and surface sterilized by rinsing in 1% Mercuric Chloride solution ($HgCl_2$) for 1 min. Then the seeds were washed thrice in distilled water and kept immersed in distilled water (as Control) and in above mentioned test solutions separately for 24 hours. The seeds were then allowed to-germinate on moist blotting papers, sterilized in Petri plates. The seeds for

Table 1 : Effects of various doses of Cadmium on seedling characters of *Pisum sativum* L.

Conc. (mg/l)	Metal (ionic state)	Germination (%)	Shoot length (cms)	Root length (cms)	Fresh Shoot wt. (mgs)	Dry Shoot wt. (mgs)	Fresh root wt. (mgs)	Dry root wt. (mgs)	Root/Shoot ratio
Control	Cd^{2+}	90.5	7.8 ± 1.2	1.5 ± 0.2	450.22	58.50	12.24	5.50	5.20
0.1		86.25	7.2 ± 1.1	1.4 ± 0.2	412.50	54.25	11.58	5.10	5.14
1.0		85.45	6.9 ± 0.4	1.2 ± 0.4	398.45	52.20	10.25	4.98	5.75
10.0		64.25	5.5 ± 1.2	0.8 ± 0.5	334.20	38.98	08.56	3.45	6.88
20.0		45.25	5.0 ± 0.4	0.6 ± 1.1	258.45	32.25	07.35	2.99	8.33
30.0		25.25	4.2 ± 1.5	0.5 ± 0.5	198.52	25.14	05.35	1.45	8.40
50.0		15.50	2.1 ± 1.2	0.3 ± 0.2	125.45	18.58	02.35	0.45	7.00

Table 2 : Effects of various doses of Copper on seedling characters of *Pisum sativum* L.

Conc. (mg/l)	Metal (ionic state)	Germination (%)	Shoot length (cms)	Root length (cms)	Fresh Shoot wt. (mgs)	Dry Shoot wt. (mgs)	Fresh root wt. (mgs)	Dry root wt. (mgs)	Root/Shoot ratio
Control	Cu^{2+}	90.50	7.8 ±1.2	1.5 ± 0.2	450.22	58.50	12.24	5.50	5.20
0.1		89.25	7.3 ±1.6	1.6 ± 0.2	445.33	56.21	12.45	5.25	4.56
1.0		86.15	7.2 ±1.2	1.5 ± 0.2	415.25	52.20	11.56	5.05	4.80
10.0		75.20	6.2 ±1.2	0.8 ± 0.3	335.25	42.52	0.8.25	3.85	7.75
20.0		72.02	5.6 ± 1.5	0.7 ± 0.5	301.25	35.22	07.40	3.35	8.0
30.0		60.25	5.1 ± 1.4	0.6 ± 0.4	269.45	29.50	06.35	3.05	8.5
50.0		55.25	4.5 ± 1.2	0.6 ± 0.5	245.45	25.20	06.00	2.45	7.5
100.0		32.25	3.3 ±1.2	0.4 ± 0.5	152.20	15.25	04.32	1.48	8.25
200.0		15.50	2.3 ± 1.5	0.3 ± 0.5	125.25	12.50	03.98	1.25	7.67
500.0		09.50	2.1 ± 1.2	0.2 ± 0.5	115.50	10.25	03.45	1.10	10.5

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germination were incubated in the dark at room temperature for 72 hours. The blotting papers were kept moistened at regular intervals with distilled water as well as the test solutions. The percentages of germination were calculated after why not at 48, 72 hrs 23 days 96 hour of the treatment. Shoot and root length, fresh as well as dry weight and root-shoot ratio were recorded and statistically analysed. Readings were trplicates and experiments were repeated twice.

The effects of Cadmium and Copper on seedling physiology were presented in Table-1 and Table-2 respectively. For both Copper and Cadmium, concentration of 1.0 mg/l and less than that have been proved to be insignificant. But with rise in concentration the effect became inhibitory. Plants treated with higher doses of metals (more than 100.0 mg/l of Copper and 20.0 mg/l of Cadmium) exhibited stunted growth and germination capacity decreased steadily. Root/Shoot value in case of Cadmium treatment initially decreased but increased from 1.0 mg/l upto 30.0 mg/l concentration. Again the ratio decreased at 50.0 mg/l level. In case of Copper treatment, the root/shoot value after initial decrease showed increase in higher levels. These increases were not as steady as in the case of Cabmium treatment.

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Effect of Pelleting on Vigour and Storability of Seeds in *Cenchrus glaucus* cv. CO1

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Pelleting of seeds not only improves seed ballistics but also provides a convenient carrier for nutrients and also insecticides and fungicides (Dunning *et al.*, 1985). Partial correction of the micronutrient deficiencies of the soil could also be possible through seed pelleting (Ribeiro and Sandos. 1994). Moreover pelleting provides a more regular shape, thus helping in precise planting and it facilitates a uniform field stand. Identifying proper pelleting material for every crop favours desirable plant population with good stand establishment. *Cenchrus* is a fodder grass sold in the form of fascicles having bristles that makes it difficult to handle. *Cenchrus glaucus* is raised in marginal lands and therefore needs initial vigour for establishment, which can be imparted through pelleting. It is therefore an experiment was conducted to assess the effect of pelleting and storability of pelleted seeds in *Cenchrus glaucus* cv. CO1.

Six month old fluffs of Cenchrus were treated with sulphuric acid to remove the extra appendages and then graded with specific gravity separator. Then seeds were pelleted using DAP @ 60 g Kg^{-1} seeds and $FeSO_4$, $ZnSO_4$, $CuSO_4$, $MnSO_4$ and Borax @ 10 g Kg^{-1} using 10 per cent maida gruel as adhesive. Arappu leaf (*Albizia amara*) powder @ 500 g Kg^{-1} seeds served as a filler. Following were the treatments: Control (without pelleting); Arappu leaf powder alone; DAP; DAP + $FeSO_4$; DAP + $ZnSO_4$; $FeSO_4$; $ZnSO_4$; $CuSO_4$; $MnSO_4$ and Borax.

The experiment was conducted with CRD design with four replications. The pelleted seeds were evaluated initially and three months after storage for germination (ISTA, 1990), seedling growth and vigour index (Abdul-Baki and Anderson, 1973). Data were analysed following Snedecor and Cochran (1967).

The pelleted seeds showed no deleterious effect on germination or vigour of seedlings immediately or even after three months of storage (Fig. 1). Seed germination was improved by $CuSO_4$ (7.4 per cent) and $FeSO_4$ (3 per cent) immediately after pelleting. Other treatment imparted no effect

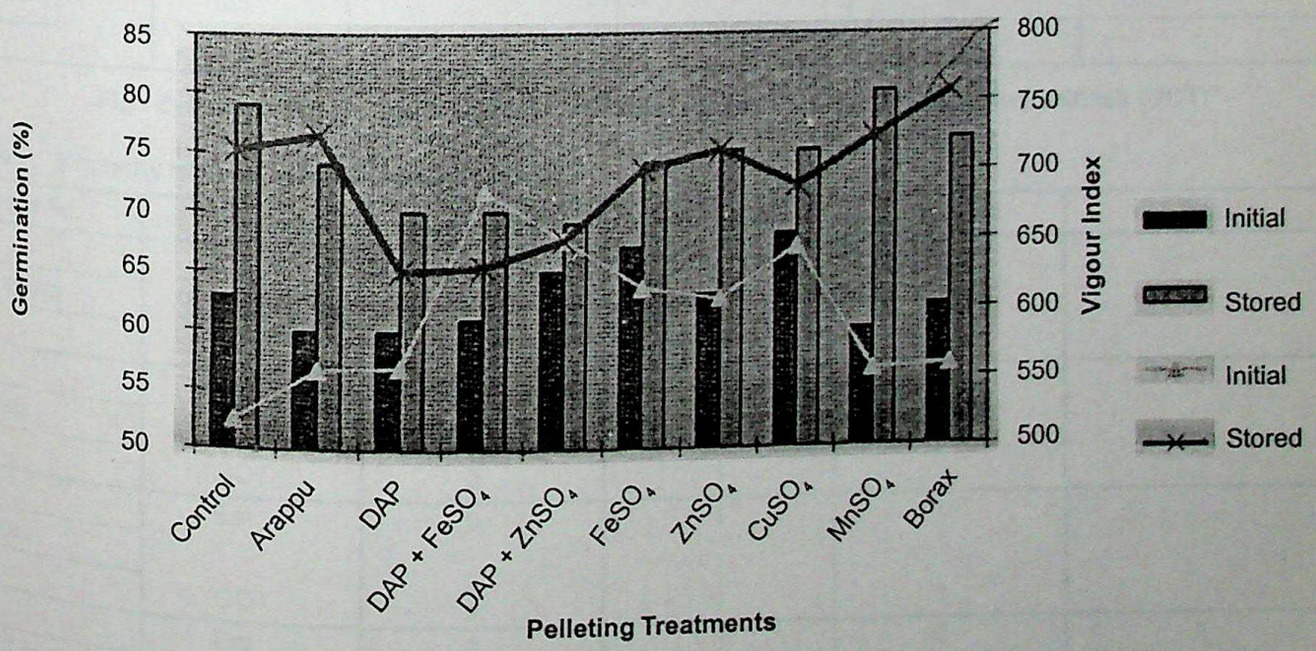

Fig. 1 Effect of pelleting on Vigour & Storability of fascicles of *Cenchrus glaucus* cv. CO1

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on the germination. Vigour index values were also higher in the seeds pelleted with nutrients particularly with DAP in combination with either $FeSO_4$ or $ZnSO_4$. Three months after storage also $MnSO_4$ recorded the highest germination (80 per cent) which was on par with control (79 per cent) followed by $ZnSO_4$ and $CuSO_4$. Pelleted seeds recorded 14 - 20 per cent more germination after three months of storage including control except DAP treated seeds since fresh seeds of cenchrus are dormant. DAP treated seeds recorded 11 per cent lower germination than control seeds. Probable reason for loss in viability of DAP coated seeds during storage could be the toxic nature of the chemical (Bapat and Umale, 1973). Other micronutrients Zn, Fe, Cu, Mn and B did not affect the seed quality. Ribeiro *et al.* (1994) stated that treatment with Zn - Bio-crop had no deleterious effect on maize seed quality but B - Bio-crop reduced germination and vigour.

Hence pelleting the scarified fluffs of *Cenchrus glaucus* using micro nutrients $FeSO_4$, $CuSO_4$ or $ZnSO_4$ @ 10 g Kg^{-1} of seeds, with arappu leaf powder as filler material, could be employed for good plant establishment.

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Study of Isoenzyme Variation in Four *Cassia* species

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At lest four species of *Cassia* are prevalent in the vast areas of India, mainly plains. Although some morphological characters have been attributed to them, but proteomic analysis are required to assign proper distinction. These four species are - *C. sophera, C. alata, C. tora* and *C. fistula*. The study of Isoenzyme pattern is based upon the differential movement of the zwitterions in electrophoretic medium. Allelic variations are responsible for the production of different forms of an enzyme, hence isoenzyme study can be considered as direct probe of gene make-up. Isoenzyme study is easier in detection for variation because of their co-dominant expression, and at the same time it is least affected by environmental pressure (Lewontin., 1974).

In the present study, analysis of two isoenzymes *viz.*- esterase (Est) and glutamate oxaloacetate transaminase (GOT) was done using vertical slab polyacrylamide gel electrophoresis (PAGE). The isoenzymes were extracted from leaves of one year old plant using extraction buffer and analysed in PAGE by standard protocol (Janarthanan and Vincent, 2007).

On the assessment of their relative mobility a total of seven band-positions were detected (three of Est and four of GOT). The most mobile band was numbered as 1 and the number increases as the mobility decreases. In the case of

Relative Mobility Value (Rm)	0	*C. sophera*	*C. alata*	*C. tora*	*C. fistula*
	0.1				
	0.2	GOT4 ▬	GOT3 ▬	GOT3 ▬	
	0.3			GOT2 ▬	
	0.4	Est3 ▬			GOT1 ▬
▬ Est	0.5		Est2 ▬	Est2 ▬	
▬ GOT	0.6				Est1 ▬
	0.7				
	0.8				

Fig : Zymogram of enzymes esterase (Est) and glutamate oxaloacetate transaminase (GOT)

Table 1 : Similarity indices of four *Cassia* species.

Name of Sp.	Enzyme	*C. sophera*	*C. alata*	*C. tora*	*C. fistula*
C. sophera	Est	1			
	GOT	1			
C. alata	Est	0	1		
	GOT	0	1		
C. tora	Est	0	1	1	
	GOT	0	1	1	
C. fistula	Est	0	0	0	1
	GOT	0	0	0	1

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isoenzyme bands of esterase (Est), Est 1 (Rm = 0.69) was observed in *C. fistula*, Est 2 (Rm = 0.59) were found for both *C. tora* and *C. alata*. Est 3 (Rm = 0.49) was observed in *C. sophera*. The enzyme glutamate oxaloacetate transaminase (GOT) was separated in four positions. GOT 1 (Rm = 0.43) was found in *C. fistula* ; GOT 2 (Rm = 0.39) was observed in *C. tora* ; GOT 3 (Rm = 0.30) were recorded in both *C. tora* and *C. alata*. GOT 4 (Rm = 0.24) was observed in *C.sophera*. From the above mentioned values it has been evident that both *C. tora* and *C. alata* might have identical gene loci. A similarity index (Table-1) was calculated using method of Sokal and Sneath (Sokal and Sneath, 1963) also indicates close similarity between them. In fact the bands of GOT in *C. tora* (GOT2 and GOT3) occupies intermediate positions between GOT1 (Rm = 0.43) and GOT4 (Rm = 0.24); thus it may be concluded that some amount of its genome had been contributed from *C. alata* and *C. fistula*. Similar study was done by Abideen and Vijayakumar (Abideen and Vajayakumar, 2002) and similarity in genetic make-up in different species of acacias was reported.

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Co-inoculation of *Pseudomonas*-PVK23 and *Rhizobium*-RVK21 Enhanced Plant Growth Activity of Peanut (*Arachis hypogaea* L.)

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Plant growth promoting rhizobacteria (PGPR) enhance plant growth and it's biological control activity protect plant from pathogenic fungi (Deshwal *et al.*, 2003). Deshwal *et al.*, (2006) reported the long-term effect of *Pseudomonas aeruginosa* GRC_1 on yield of subsequent crops of paddy after mustard seed bacterization. Recently, Mishra *et al.*, (2009) reported that co-inoculation of *Bacillus thuringiensis*-KR1 with *Rhizobium leguminosarum* enhanced plant growth and nodulation of pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.).

In present study, twenty five *Pseudomonas* strains were isolated from cultivated soil and twenty five *Rhizobium* strains were isolated from the nodules of gram plant. Both *Pseudomonas* and *Rhizobium* strains were characterized on the basis of Holt *et al.*, (1994). Further, interaction study had been done according to Deshwal *et al.* (2003). Both strains *Pseudomonas*-PVK25 and *Rhizobium*-RVK21 produced indole acetic acid (IAA), siderophore, HCN, phosphatase enzyme and both strains did not mutually inhibit the growth of each other. Therefore, an experiment was conducted to analyse the effect of co-inoculation of *Pseudomonas*-PVK23 and *Rhizobium*-RVK21 on growth of peanut.

Earthen pots were filled with soil and sterilized as mentioned by Deshwal *et al.*, (2003). Seeds bacterized with *Pseudomonas*-PVK23 and *Rhizobium*-RVK21 along with their respective non-bacterized seeds (control) were sown in four sets of treatment: treatment I - soil inoculated with *Pseudomonas*-PVK23; treatment II- soil inoculated with *Rhizobium*-RVK21; treatment III - soil inoculated with *Pseudomonas*-PVK23 and *Rhizobium*-RVK21; treatment IV - uninoculated soil as control (with non-bacterized seeds). Three seeds per pot were sown and after 15 days, thinning was done to raise only single healthy plant in each pot. The plants were irrigated with tap water whenever required. Seed germination (%) was noted on 15th day of sowing. Seedling biomass and nodule weight were recorded after 45 days of sowing. Doses of *Pseudomonas*-PVK23 and *Rhizobium*-RVK21 suspension (10^8 cfu ml^{-1}) were periodically applied following soil drench method at 15 and 30 days after sowing.

After 45 days, results suggested that co-inoculation of *Pseudomonas*-PVK23 and *Rhizobium*-RVK21 enhanced root colonization seed germination, shoot length, root length, fresh seedling weight, dry seedling weight and nodule number (Table 1, 2). Similarly, Askary *et al.*, (2009) reported that co-inoculation of wheat seeds with *Azospirillum brasilense* and *Rhizobium meliloti* had positive and significant effect of grain yield and further, Mishra *et al.*, (2009) reported that co-inoculation of *Bacillus thuringiensis*-KRI with *Rhizobium leguminosarum* enhanced nodule number, plant weight and total biomass. All above study showed that co-inoculation of *Pseudomonas*-PVK23 and *Rhizobium*-RVK21 enhanced plant growth activity of peanut.

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Table 1 : Root colonization study of *Pseudomonas*-PVK23, *Rhizobium*-RVK21, and Co-inoculation of *Pseudomonas*-PVK23, *Rhizobium*-RVK21 on Peanut (Arachis) after 30 and 45 days of sowing.

S. No.	Treatment	Population ($x10^5$)	
		30 days	45 days
1.	*Pseudomonas*-PVK23	2.2 ± 0.17	4.3 ± 0.16
2.	*Rhizobium* - RVK21	2.6 ± 0.18	4.9 ± 0.17
3.	*Pseudomonas* - PVK23 + *Rhizobium* RVK21	3.9 ± 0.17	6.1 ± 0.19
4.	Control	0	0

Values are means of twenty replicate ± SD

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Table 2 : Effect of bacterization with *Pseudomonas*-PVK23, *Rhizobium*-RVK21 and co-inoculation of *Pseudomonas*-PVK23 *Rhizobium*-RVK21 on the growth of peanut (*Arachis*) after 45 days of sowing.

S.No.	Treatment	Seed germination	Length (cm)		Fresh weight (gm) Seedling	Dry weight (gm) Seedling	Nodules (No)
			Shoot	Root			
1.	*Pseudomonas*-PVK23	80**	19.8**	7.0**	12.24**	5.39**	0
2.	*Rhizobium* - RVK21	85**	19.2**	7.3**	11.73**	5.35**	43**
3.	*Pseudomonas* - PVK23 + *Rhizobium* RVK21	90**	28.3**	9.2**	18.54**	9.08**	52**
4.	Control	60	8.3	3.2	3.70	1.443	0

Values are mean of twenty replicates,**, significant at 0.01 levels of LSD as compared to control.

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In Vitro Organogenesis and Enhanced Somatic Embryogenesis of *Solanum nigrum* L., using Leaf Disc as Explant

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An experimental study was performed during 2008-2009 to assess the totipotency of *Solanum nigrum* L, which was expressed through organogenetic as well as somatic embryogenetic responses. Benzyl amino purine (BA) was found to be more potent than Kinetin for shoot regeneration. whereas multiple microshoots sprouting was favoured by BA in association with naphthalene acetic acid (NAA) and lower dose of Thidiazuron. Embryogenic callii were induced in a medium containing MS basal plus 2 4-D. Globular stage followed by heart and torpedo in MS basal plus BA, Kinetin (Kn) and Gibberellic acid (GA_3). Salinity tolerant cell lines were screened under selective pressure with different concentrations of sodium chloride (NaCl) and potassium chloride (KCl). Young leaf as explant scored better.

Keywords : *Solanum nigrum*, Organogenesis, Salt stress, Somatic embryogenesis, Thidiazuron, Totipotency.

INTRODUCTION

Solanum nigrum L. is an annual herb, growing throughout India especially in alluvial planes. This plant is known to have medicinal properties like antiperiodic, febrifuge, antiphlogistic, diaphoretic, diuretic, emollient, purgative as well as sedative (Grieve,1984). The whole plant, mainly the leafy part is used as decoction in dropsy, enlargement of liver and in jaundice. The syrup is prescribed as expectorant. The leaf juice is given in inflammation of kidneys bladder, also useful against gonorrhea and piles (Pandey, 2007). Recent findings showed that this particular plant possesses steroidal saponins, such as solanigrosides and degalactotigonin which are potent antineoplastic agents (Zhou *et. al.* 2006; Hu *et. al.,* 1999). Anti inflammatory and antiallergic property of the plant might be due to glycoprotein (Lim, 2005). Micropropagation techniques are nowadays preferred over conventional methods of propagation due to possible development of pathogen free plants under aseptic conditions. Moreover, the desired plant organs and the active principles could be quickly harvested if proper organogenesis is programmed. Tissue culture methods were used in *in vitro* selection of salt tolerant cell lines (Ben-Heyyim and Goffer, 1989; Srivastava and Khare 2004).

MATERIAL AND METHODS

Young leaves were selected as explants and properly washed then surface sterilized with 0.1% $HgCl_2$ for 5 min, rinsed thrice with double distilled water. Leaf disc of 5 mm diameter were cultured with abaxial surface on Murashige and Skoog's basal medium (Murashige and Skoog, 1962), supplemented with various concentrations of different plant growth regulators, either singly or in combinations. Cultures were incubated at 24 ± 2ºC temperature, 70% Relative humidity and 16 hour photoperiod (under cool white fluorescent light of 1600 Lux for 16 hours a day). Treatments were replicated thrice and each replicate consisted of 5 cultures. BA (1 mg/l, 2 mg/l and 4 mg/l); Kn (1 mg/l, 2 mg/l and 4 mg/l); and other combinations like BA plus IAA, BA plus NAA, Kn plus IAA, Kn plus NAA and BA and Kn together with NAA etc. were used along with MS basal. MS basal fortified with 1.0 mg/l BA, 0.5 mg/l NAA and 0.5 mg/l Thidiazuron (TDZ) found as efficient as a composition having MS basal plus 4.0 mg/l BA, 1.0 mg/l NAA in callusing. For somatic embryo development, embroygenic callii were first induced using MS basal supplemented with 1 mg/l 2, 4-D and 0.5 mg/l IBA, followed by subculturing in MS basal supplemented with either 2 mg/l BA and 0.5 mg/l GA_3 or 2 mg/l BA, 0.5 mg/l Thidiazuron (TDZ) and 0.5 mg/l Gibberelic acid (GA_3). The later combination of culture medium was observed to be more potent for inducing proliferative nature of the already induced somatic embryo without any *in vitro* screening. Equal sized leaf explants were inoculated in the first medium containing MS basal salts fortified with 1 mg/12, 4-D and 0.5 mg/l IBA. Two salts- Sodium chloride (NaCl) and Potassium chloride (KCl) were tested for *in vitro* salinity tolerant cell lines. The concentration series was: 0.1, 0.5, 1.0, 2.0, 5.0, 10.0 g/l. The surviving callii were sub-cultured in the medium having composition of MS basal supplemented with 2 mg/l BA, 0.5 mg/l Thidiazuron (TDZ) and 0.5 mg/l gibberelic acid (GA_3). As against in the prior case of organogenesis where unipolar axis development took place, in the later part of the experiment for somatic embryogenseis, bipolarity in terms of axial development was observed. At all the stage the axenic conditions were practised and the cultures were maintained at the already mentioned physical conditions.

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Table 1 : Effect of plant growth regulators on callus induction, microshoot formation and shoot regeneration of *Solanum nigrum* L.

Conc. of plant growth regulators* (mg/l)	Explants showing callus Induction #	Number of microshoot per explant #	Number of shoot per explant #
Control {MSO}	-	-	02.00 ± 0.00
BA (1.0)	38.20	04.41 ± 0.020	-
BA (2.0)	68.62	08.20 ± 0.032	03.18 ± 0.015
BA (4.0)	52.82	03.60 ± 0.045	-
Kn (1.0)	18.10	06.32 ± 0.090	-
Kn (2.0)	29.62	08.58 ± 0.060	-
Kn (4.0)	60.22	05.12 ± 0.030	-
BA (2.0) + NAA(1.0)	86.21	10.15 ± 0.045	08.12 ± 0.042
BA (4.0) + NAA (1.0)	70.42	08.16 ± 0.080	05.20 ± 0.035
BA (2.0) + IAA (1.0)	38.42	10.26 ± 0.060	06.22 ± 0.048
BA (4.0) + IAA (1.0)	20.50	03.12 ± 0.030	-
Kn (2.0) + NAA (1.0)	32.00	04.41 ± 0.020	-
Kn (4.0) + NAA (1.0)	48.22	09.90 ± 0.030	05.21 ± 0.045
Kn (2.0) + IAA (1.0)	36.40	11.00 ± 0.075	04.12 ± 0.030
BA (2.0) + Kn (1.0 + IAA (1.0)	28.28	08.20 ± 0.045	15.21 ± 0.075
BA (2.0) + Kn (1.0 + NAA (1.0)	20.18	04.10 ± 0.030	06.68 ± 0.075
BA (1.5) + NAA (0.5) + TDZ (0.5)	80.50	12.05 ± 0.045	-

MSO= Murashige and Skoog's basal medium,* = supplemented in MSO, # = mean, ± = standard error (5 replicates repeated thrice).

Table 2 : *In Vitro* root formation from leaf derived callii of *Solanum nigrum* L.

Conc. of plant growth regulators* (mg/l)	Percentage of root formation
Control 1 (MSO)	0.00
Control 2 (0.5 MSO)	2.5
IBA (0.5)	55.0
IBA (1.0)	80.0
IAA (1.0)	45.0
NAA (1.0)	7.5

MSO = Murashige and Skoog's basal medium,* supplemented in MSO.

RESULTS AND DISCUSSION

The efficiency of organogenesis in case of young leaf as explant was found to be more than other explants for the plant under study. Apart from leaf other part of the plant were used as explants by several investigators (Jahan and Hadiuzzuaman,, 1996); even protoplasts were used as starting materials (Szczerbakowa *et.al.,* 2000). Among the various plant growth regulators used. Benzyl aminopurine (BA) showed more efficacy towards callus induction, compared to corresponding concentrations of Kinetin (Kn). MS basal supplemented with 4 mg/l BA was found to be suited for better callus induction. MS basal supplemented with 2 mg/l BA and 1 mg/l Naphthalene acetic acid (NAA) favoured microshoot generation better than that in 5 mg/l Kinetin and 2 to 3 mg/l NAA. Apart from NAA, Indole acetic acid (IAA) responded too but with much less efficiency (Table1). When Indole butyric acid (IBA) was added along with BA or Kinetin, more compact green callii were induced. MS basal supplemented with BA or Kinetin and NAA or IAA developed brownish friable callii (Jawahar *et al.,* 2001). In another composition MS basal fortified with 1.0 mg/l BA, 0.5 mg/l NAA and 0.5 mg/l Thidiazuron (TDZ) found as efficient as a composition having MS basal plus 4.0 mg/l BA, 1.0 mg/l NAA in callusing. The microshoots developed in MS basal supplemented with 2 mg/l BA, and 1 mg/l NAA were subcultured in MS basal supplemented with 2 mg/l BA, 1 mg/l Kinetin and 1 mg/l IAA to get better response of shoot regeneration (Table 1). Root development was found to be maximum in 0.5 MS basal supplemented with 1 mg/l IBA (Table 2). Similar results were reported by others also (Gajbhiye *et.al.,* 2006). When the plant growth regulators were used singly, BA in lower dose (at 1 mg/l and 2 mg/l) was found to be more potent in callus development than corresponding concentrations of Kinetin. But at higher sose at 4 mg/l Kinetin was found to be more potent. When NAA was added with MS plus BA and Kn separately, then the rate of callus development and also shootler production were increased. Again it was also observed that, TDZ was capable to induce callus more effectively at comparatively lower dose. This finding was in accordance with other observations by several authors (Dhawan *et.al.*, 2004;Qu Li *et.al.*, 2006). Again it was observed that the higher the concentration of the salt, the more was the time taken for callus initiation (Table 3).

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Table 3 : Callusing response of leaf explants of *Solanum nigrum* L.under different doses of salts.

Salt (g/l)	NaCl			KCl		
	DTI	% response	% survival	DTI	% response	% survival
0 (control)	3 – 4	100	100	3 – 4	100	100
0.1	4 – 5	70	55	4 – 5	65	68
0.5	4 – 5	75	58	4 – 5	70	65
1.0	4 – 5	80	60	4 – 5	75	75
2.0	4 – 5	75	45	6 – 7	75	65
5.0	7 – 8	85	40	8 – 9	75	45
10.0	8 – 9	90	25	9 – 10	88	45

DTI = days till initiation.

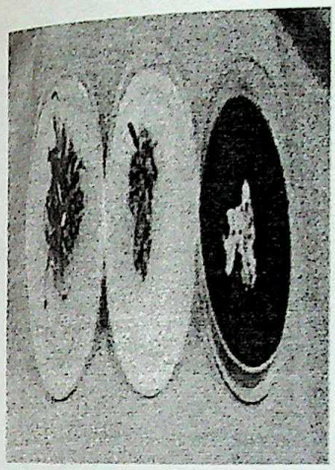

Fig 1 : Callus in BA (2.0) & NAA (1.0) (without salt treatment).

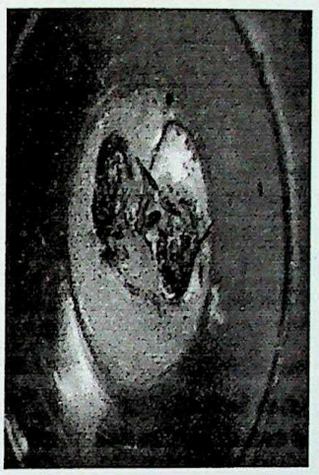

Fig 2 : Plantlet regeneration from embryogenic callus (without salt treatment).

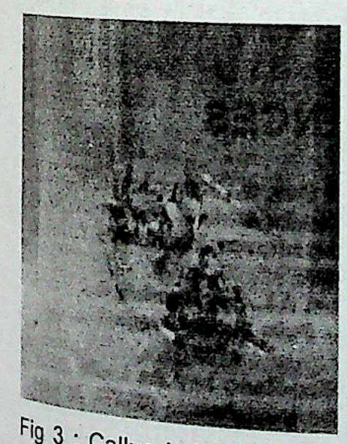

Fig 3 : Callus induced under 1.0 g/l NaCl.

Fig 4 : Plantlet regeneration from embryogenic callus (treated with salt). Left to right : 0.5; 1.5 g/l NaCl & 1.0; 0.1 g/l KCl respectively.

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EMS Induced Biochemical Variations in Cultured Cells of *Vigna radiata* L.

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In-vitro induction of mutations for qualitative and quantitative changes of bioactive compounds is an interesting area in plant metabolic research. In present investigation, *in vitro* mutagenesis of *Vigna radiata* cell culture was studied using chemical mutagen, Ethyl Methyl Sulphonate (EMS). Cultured cells were grown in MS medium supplemented with 0.01% and 0.05% concentration of EMS. Seeds treated with similar doses of EMS were grown separately in MS medium for parallel study. Two weeks old cultured cells were used as a sampling material for the analysis of protein (Lowry method) and saponin compounds by HPTLC method. The protein estimate in EMS treated cell sample showed quantitative reduction (*i.e.,* 4.87 and 3.67 mg/g) in 0.01 and 0.05% EMS compared to control estimate *i.e.,* 10.80 mg/g. Quantification of saponins was done by using HPTLC method. Saponin content in treated seedlings was observed to be more as compared to control, whereas in callus it was observed to be more in 0.01% of the treated callus as compared to 0.05% and control. The *Vigna* seedlings grown from treated seeds under *in-vitro* condition also indicates variations in protein content and secondary metabolic product *i.e.,* saponin. The present study thus indicates the impact of EMS under *in vitro* condition by inducing biochemical variations in studied material.

Keywords : *Vigna radiata*, Callus, EMS, Saponin, Protein.

INTRODUCTION

Plant tissue culture technology for micro propagation, virus elimination & genetic engineering has made a remarkable progress in the recent years. Variability is highly essential to allow a breeder to select cultivars of plants which are more adapted to environmental changes, more efficient in utilizing nutrients,, more tolerant to diseases and pests and improved in yield and quality. The more important factors in producing variability in plants are hybridization. recombination and mutation (spontaneous or induced). However, the natural occurring mutation rate is too low for practical applications. Therefore, physical and chemical mutagens have proven usefulness for increasing the frequency of variations and mutations. Application of chemical mutagens to various *in vitro* systems may offer several advantages, if applied under well-defined conditions. Efficient mutagenesis of plant cell cultures is possible, as the cells can be uniformly treated with physical or chemical mutagens. Fundamental and applied aspects of experimental mutagenesis have been extensively reviewed (Damodaran *et al.* 2007; Van, 1998).

There are only a handful of chemical mutagens which are applied to plant cell cultures for induction of mutations under *in vitro* condition. The most commonly used has been ethyl methane sulphonate (EMS). It produces random mutations in genetic material by nucleotide substitution, specifically by guanine alkylation. This typically produces only point mutations. Such random mutations may produce changes in gene sequence which may alter primary and secondary metabolite production (Luan *et al,* 2006; Svetleva & Crino,2005).

In vitro induction of mutations for qualitative and quantitative changes of bioactive compounds is an interesting area in plant metabolic research. In present investigation, the preliminary *in-vitro* studies were conducted for *in vitro* mutagenesis in *Vigna radiata* cell culture by using chemical mutagen, EMS.

MATERIAL AND METHODS

In vitro grown seedlings obtained from sterilized seeds grown on Murashige and Skoog medium were used as control. Similarly seedlings were obtained from the treated seeds (EMS 0.01% and 0.05% for 2 hours). Seedlings were then analyzed for protein and saponin content.

EMS treatment to Sub-cultured callus : Leaf and stem explants from 7 days old *in-vitro* grown seedlings (without EMS) were used for the induction of callus. MS medium supplemented with 2, 4-D+Kinetin, 2, 4-D+BAP and 2,4-D+Kinetin+BAP (see Table1) was used for callus induction. The better callus induction was observed in the media supplemented with the 2, 4-D+BAP (1.0+0.1 mg/L). Well grown callus was subcultured on MS medium supplemented with two concentrations of EMS (0.01% and 0.05%). Separate sub-cultured flask without EMS was maintained as control. Constant observations were taken to observe the growth of sub-culture

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Table 1 : Growth of *Vigna radiata* (L.) callus cells in different hormonal combinations.

Sr. No	Growth regulators	Concentrations (mg/L)	Growth of callus			
			7 days	14 days	21 days	28 days
1.	2,4- D+Kin	0.5 + 0.5	+	+	++	++
		1.0 + 0.5	+	++	++	+++
		1.5 + 0.5	+	++	++	++
		2.0 + 0.5	+	++	+++	++
2.	2,4-D+BAP	0.5 + 0.5	+	++	++	+++
		1.0 + 0.5	+	++	++	+++
		1.5 + 0.5	+	++	++	++
		2.0 + 0.5	+	++	++	+++
3.	2,4-D +Kin+BAP	0.5 + 0.5 + 0.1	+	++	+++	+++
		1.0 + 0.5 + 0.1	+	++	++	+++
		1.5 + 0.5 + 0.1	+	++	++	++
		2.0 + 0.5 + 0.1	+	+	++	++

+ Initiation of callus ++ Good amount of callus; +++ Very good amount of callus.

Table 2 : Total protein content in seedlings and sub-cultured callus treated with EMS.

Experimental	Sample	Protein concentration in mg/g
14 days old sub-cultured callus cells	Control	10.80
	0.01%	4.87
	0.05%	3.67
Seedlings	Control	7.83
	0.01%	6.28
	0.05%	4.58

tissue in varying concentrations of EMS and in control flask for two weeks. Both these calli were analyzed for estimation of protein and saponin.

Extraction and estimation of protein : The extraction and estimation of proteins was carried out by using the method of Lowry *et al.* (1951). Calculation was done using BSA calibration curve.

Extraction, identification and quantification of Saponin : (Ramavat and Merillion, 1999) 2 gm of powdered calli and seedlings (control as well as treated) were extracted with 10 ml of 70% ethanol for 10 minutes by refluxing. The ethanolic filtrate was taken out and evaporated to 5 ml. 3 ml of this extract was taken and mixed with 5 ml of water saturated n-butanol. The mixture was shaken thoroughly. The saponin absorbed in the butanol phase was separated with the help of separating funnel and concentrated to about 2 ml. The identification of saponins were done by TLC using the chloroform: glacial acetic acid: methanol: water (64:32:12:8) as a mobile phase. Dried silica gel plate was sprayed with anisaldehyde-sulphuric acid reagent which was followed by heating up to 100ºC. Saponins were detected in the form of brown spots as appeared on silica plate. The plate was scanned in HPTLC quality evaluation instrument for quantification. The quantification was done by

Table 3 : Total Saponin content in *Vigna radiata* seedling and sub-cultured callus treated with EMS.

	Saponin content in Seedling (µg / g)	Saponin content in Callus (µg / g)
Control	13.3	6.9
0.01%	17.5	7.2
0.05%	20.0	3.6

considering area of retention for different saponins as appear in HPTLC graph.

RESULTS AND DISCUSSION

In the present investigation, *in-vitro* mutagenesis has been carried out in cell cultures of *Vigna radiata* by using chemical mutagen, EMS.

On the basis of results obtained in the present work it can be concluded that the selected plant system, *Vigna radiata* responds very well for the callus induction in the hormonal combination *i.e.*, 2,4-D : BAP (0.5 + 0.1 and 1.0 + 0.1 mg/L) (see Table- 1; Fig- 1 and 2).

Protein was estimated using the method of Lowry *et al.* (1951). In sub-cultured callus, protein content in control (without EMS) sub-cultured cells was estimated to be 10.80 mg/g. In the sub-cultured cells treated with 0.01% EMS conc., the protein content was reduced to 4.87 mg/g whereas, in 0.05% EMS treated sub-cultured cells the content was further reduced to 3.67 mg/g. In case of seedlings, the protein content in control seedlings was 7.83 mg/g. In 0.01% and 0.05% EMS treated seedlings. the protein content was 6.28 mg/g and 4.58 mg/g respectively (Table-2). It indicates the impact of chemical mutagen at biochemical level. The studies doses of EMS may have interfered the metabolism at enzymatic level.

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Fig. 1 : 21 days old Callus of *Vigna radiata*.

Fig. 2 : Sub-cultured callus of *Vigna radiata* from leaf explant

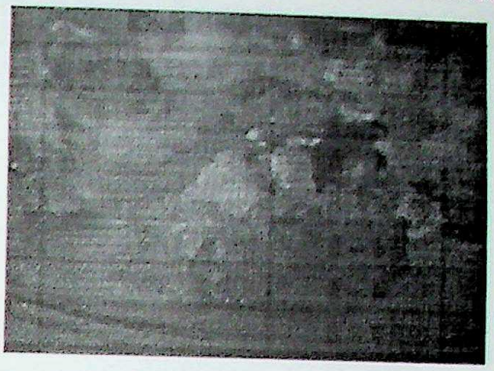
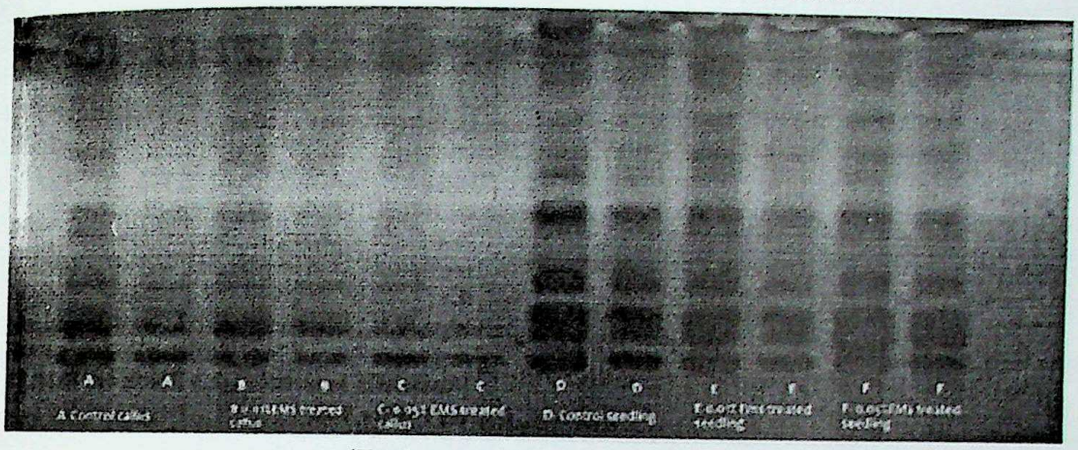


Fig. 3 : HPTLC Image @ 366 nm after Derivatisation:



(Each sample was run twice)

A: Control callus B: 0.01% EMS treated callus C: 0.05% EMS treated callus D: Control seedling E: 0.01% EMS treated seedling F: 0.05% EMS treated seedling

Quantification of saponin is carried out by a HPTLC (High Performance Thin Layer Chromatography, Fig-3). The total saponin content in control sub-cultured cells was estimated as 6.9 µg/g. In 0.01% EMS treated sub-cultured cells it was increased to 3.6 µg/g and in 0.05% EMS treated sub-cultured cells it was 7.2 µg/g. The total saponin content in control seedling was estimated to be 13.3 µg /g. In 0.01% EMS treated seedling it was increased and was 17.5 µg/g and in 0.05% EMS treated seedling it was estimated 20.0 µg/g (Table-3). It indicates that, the studied EMS concentration causes variation in total saponin content. The saponin content was increased in 0.01% EMS treated sub-cultured cells but decreased in 0.05% EMS treated sub-cultured cells when compared to control calls sample. In seedling sample, the total saponin content indicates dose dependency with EMS concentration.

In present investigation, EMS treatment induced reduction in growth characteristics of *Vigna radiata* by decreasing protein content in totality. It may be contributed to the damage produced at physiological level by the studied mutagenic doses. The *Vigna* seedlings grown from treated seeds under *in-vitro* condition also indicates variations in protein content and secondary metabolic product *i.e.*, saponin. The secondary metabolic product *i.e.*, saponin was seen to be altered by the mutagenic treatment with EMS. In this context, it can be concluded that, ethyl methane sulphonate (EMS), although is mutagenic agent, is proven to be effective, under *in vitro* condition and produces variation in primary and secondary metabolite products.

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An Efficient *In Vitro* Plant Regeneration System for *Bacopa monnieri* (L) - Pennel

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An ideal protocol for rapid and large-scale *in vitro* propagation of *Bacopa monnieri* (L.) Pennel (Vernacularly known as Nirbrahmi in Marathi) is described. *Bacopa* is a valuable medicinal plant used as brain tonic and analgesic. Leaf explants obtained from field grown plants of *Bacopa* were tested for callus induction on Murashige and Skoog's (MS) medium, supplemented with different concentrations of 2,4-D, ranging from 0.5 - 4.0 mg/l and combination with 2 and 4 mg/1 BAP. The explants, cultured in MS medium, supplement with 2 mg/l 2,4-D in combination with 4 mg/1 BAP, produced profuse and healthy callus. When the callus was subcultured, maximum number of multiple shoots (18 ± 0.13) were obtained on MS medium supplemented with BAP (4 mg/L) and IAA (2 mg/L). The shoots were transferred to a rooting medium which consisted of MS medium supplemented with either IBA and BAP. It was observed that IBA at a concentration of 4 mg/1 produced maximum number of roots. Rooted plantlets were successfully established in the field after hardening.

Abbreviations : 2,4-D: 2,4-dichlorophenoxyacetic acid, BAP: 6- Benzyl amino purine, IBA: Indole Butryric acid, IAA: Indole acetic acid.

Keywords : *Bacopa monnieri*, Nirbrahmi, Adventitious shoots.

INTRODUCTION

Brahmi *(Bacopa monnieri* L.*)* Pennel, a member of Scrophulariaceae is an important medicinal plant found in humid and warmer parts of the world, growing in damp or marshy areas (Anonymous, 1948). In Ayurveda the plant is used for anti-inflammatory, analgesic, antipyretic, anticancer and antioxidant activities (Tripathi *et al.,* 1996, Vohora *et al.,* 1997). The medicinal properties of *B. monnieri* have been attributed to the presence of different types of saponins, for *e.g.,* Bacosides A, B, C and D (Rastogi *et al.,* 1994). The naturally occurring plants show great environmental variability and there is an increasing demand for *in vitro* propagated plants which are genetically and morphologically uniform. To fulfil the demand for morphologically uniform plants and to achieve large-scale propagation of these plants in a short period, we have undertaken the present investigation of establishing an ideal protocol for rapid and large-scale *in vitro* propagation of *Bacopa*. This would speed up the production rate of the average plants by approximately 10,000 times and a large number of productive plants can be multiplied routinely through tissue culture. The aim of our present study is to enhance the ability and fastness of shoot regeneration from apical meristem of *B. monnieri*.

MATERIAL AND METHODS

The disease free, young apical meristem explants were selected and were washed under running tap water for 30 minutes. Then they were washed in an shake up solution of liquid detergent 2% (v/v) Teepol for two minutes and rinsed in distilled water for three times. Surface sterilization was performed by immersion of the explants in 70% (v/v) aqueous ethanol for 40 seconds followed by 0.2% (w/v) mercuric chloride for three minutes. Finally, the material was thoroughly rinsed with sterile distilled water for two times to remove the traces of mercuric chloride. All the explants were cut into pieces approximately of 10-15 mm long for inoculation under aseptic conditions.

Culture conditions : The media used were based on MS formulation (Murashige and Skoog, 1962), containing 3% (w/v) sucrose and 0.8% (w/v) agar. The pH of the media was adjusted to 5.8 and autoclaved at 121°C for 15 minutes at 15 psi pressure. The explants were placed in culture tubes and bottles containing 20 ml of media with respective growth regulatiors. The cultures were incubated at 25 ± 2° C under 16/8 hours (light/dark) photoperiod and relative humidity of 50-60%.

Callus induction : The explants were cultured on MS medium supplemented with various concentrations of plant growth regulators including 0.5, 1, 2, 3 and 4 mg of 2,4-D in combination with 2 and 4 mg BAP.

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Shoot regeneration: The callus was subcultured for shoot regeneration on the medium supplemented with various concentrations and combinations of 2 or 4 mg BAP and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 or 4 mg IAA.

Root induction: The shoots were excised from regeneration medium and were transferred to MS medium supplemented with different concentration and combinations of plant growth regulators including 0.5 or 4 mg IBA and BAP.

Acclimatization : Rooted plantlets were gently washed with running tap water followed by sterile distilled water and then successfully transferred to pots containing sterile garden soil. The pots were covered with porous polyethylene bags for maintaining high humidity. The hardened plants were maintained in the culture room. The polyethylene bags were removed after 2 weeks and then the hardened plantlets were successfully transferred to net house for further growth and development.

Statistical analysis: Experiments were set up systematically and each experiments was replicated thrice. Percentage of number of shoots per explants, number of roots per shoot and root length were recorded. Means and standard errors were carried out for each treatment.

RESULTS AND DISCUSSION

The callus induction usually required the presence of auxins or cytokines in the nutrient medium. The explants of apical meristem are used for callus initiation. These were inoculated on the MS medium having different combinations and concentrations of BAP and 2,4-D. The callus formation stared after 15 to 20 days at the end of explants (Table-1). The

Table 1 : Effect of different culture media on contamination, regeneration and appearance of callus *in vitro*.

MS Medium + PGR	Contamination	Regeneration	Appearance
MS+0.5 2-4 D+2.0 BAP	No contamination	No Regeneration	Jelly type green
MS+1.0 2-4 D+2.0 BAP	No contamination	Regenerations	Globular
MS+2.0 2-4 D+4.0 BAP	No contamination	Regenerations	Globular pale yellow
MS+3.0 2-4 D+2.0 BAP	No contamination	Regenerations	Globular

*Data represent mean of 10 replicates per treatment in three repeated experiments.

Table 2 : Effect of various media on multiple shoot formation of the Apical meristem of *Bacopa monnieri*.

MS + Growth regulator (mg/l)		No. of shoots (Average)	Avg. shoot *length (cm)
BAP	IAA	Apical meristem	Apical meristem
2	0.5	10 ± 0.60	3.2 ± 0.22
2	1.0	12 ± 0.12	4.3 ± 0.55
2	1.5	14 ± 0.30	3.7 ± 0.13
4	**2.0**	**18 ± 0.13**	**5.3 ± 0.22**
2	**2.5**	**15 ± 0.14**	**4.0 ± 0.14**
4	3.0	11 ± 0.50	4.7 ± 0.11
4	3.5	12 ± 0.50	4.8 ± 0.14
4	4.0	12 ± 0.20	4.1 ± 0.23

* Data represents mean of 10 replicates per treatment in three experiments.

Table 3 : The effect of various media on root induction *in vitro* shoot regeneration of *Bacopa monnieri*.

MS + Growth Hormone (mg)		Number of Roots* (cm)	Average length of Root. *(cm)
IBA	BAP		
0.5	1.0	1.2 ± 0.20	1.2 ± 0.22
1.0	2.0	1.5 ± 0.22	0.7 ± 0.30
2.0	3.0	1.8 ± 0.70	1.5 ± 0.40
3.0	4.0	2.0 ± 0.00	1.0 ± 0.60
4.0	**0.0**	**3.50 ± 0.55**	**2.7 ± 0.44**
0.0	2.0	2.7 ± 0.11	2.0 ± 0.15
3.0	0.0	2.0 ± 0.22	1.3 ± 0.35
0.0	1.0	1.0 ± 0.10	1.5 ± 0.50
2.0	0.0	2.70 ± 0.40	1.7 ± 0.12

* Data represents mean of 10 replicates per treatment in three repeated experiments.

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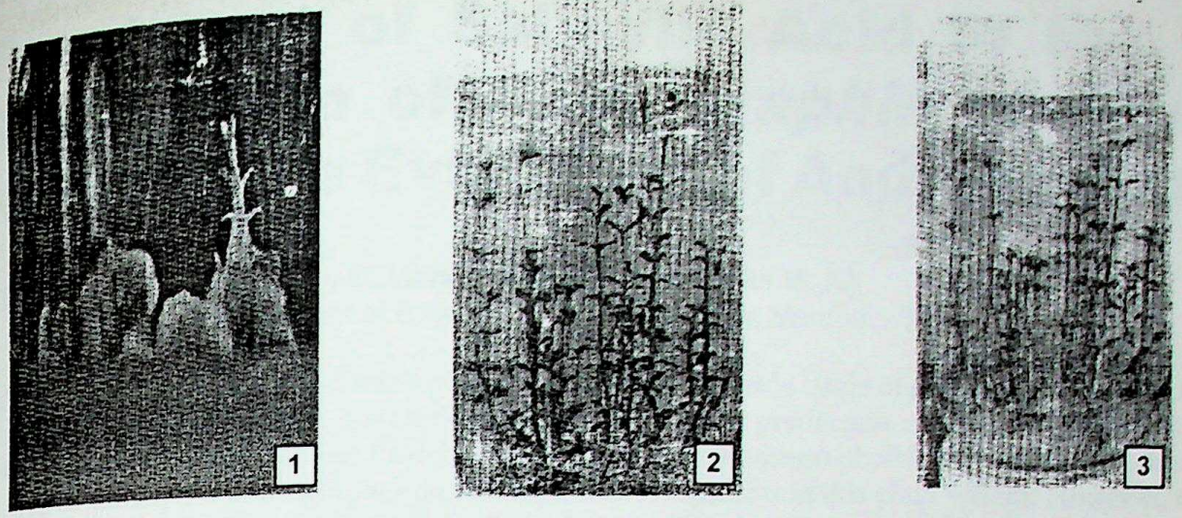

Fig. 1-3, *Bacopa monnieri*, 1, Callus and shoot formation; 2, Shoot formation; 3, Root formation,

globular callus was initially yellow in colour but later it changed to green. The explants, grown on MS medium supplemented with 2 mg/l 2, 4-D and 4 mg/l BAP showed rapid and more amount of callus growth (Fig-1). A similar result was observed by Debanath *et al.*, (2006).

The shoot regeneration was obtained on MS medium supplemented with various concentrations and combinations of BAP and IAA. Among the BAP and IAA, the former was found to be superior and more effective, in shoot induction development (Table-2). Maximum shoot bud induction (18 ± 0.13) and shoot length (5.3 ± 0.22) was obtained on MS medium with BAP (4.0 mg/L) and IAA (2.0 mg/L). The present study of shoot formation by the use of MS medium with 4 mg/l BAP and 2 mg/l IAA further increase in shoot formations within 15-18 days (Fig.2). Similar results were also reported in Niger (Nikam and Shitole, 1993) and shoot regeneration potentional of IAA has also been repoted by Tejavathi and Shailaja (1999) in *Bacopa monnieri*. The promotion of the shoot regeneration also observed by the positive effect of BAP in combinations with IAA several medicinal plant species (Manickam *et al.*, 2000).

The long shoots were cut out from each regeneration medium and transferred to MS medium supplement with 0.5 to 4 mg/l IBA and BAP (Table-3). IBA was found to be the best rooting hormone than BAP in *Bacopa* and maximum root induction (3.50 ± 55) was observed on MS medium with 4.0 mg/l IBA (Fig.3). Tiwari *et al.*, (1998) stated that higher concentrations of IBA and IAA in the media caused increase in length of the roots. Similar effect of IBA on *in vitro* rooting of regenerated shoots has also been reported in *Centella asiatica* (George *et al.*, 2004).

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Treatment of Salicylic Acid to the Cell Suspension of *Andrographis paniculata* Nees for the Evaluation of Andrographolide

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Andrographis paniculata Nees. is a well known medicinal plant for its wide range of medicinal properties. Plant tissue culture techniques have been used for micro propagation and production of secondary metabolites at *in vitro* level. The majar terpenoids *viz*, 14-deoxy andrographolide, andrographolide, neoandrogapholide and 14-deoxy-11, 12-didehydro andrographolide are the active constituents in this plant system. However, these compounds are found to be absent in cultured cells of *A. paniculata* when grown in MS medium supplemented with 2, 4-D and BAP. Several possible factors have been determined for the absence of andrographolide in callus culture previously. According to our previous study, the field grown plant shows the spectral peak for andrographolide but the cultured cells do not indicate the presence of this compound. In this study, salicylic acid with varying concentration (0.05 mM, 0.5 mM and 1.5 mM for 24 hrs) was used to trigger the synthesis of andrographolide in suspension culture of *A. paniculata*. The HPTLC analysis of all the three treatments for the studied time duration reveals marked variations in total number of peaks for unknown compounds compared to control but all the three concentrations lacking spectral peak for known compound *i.e.*, andrographolide in particular comparable to standard andrographolide compound. The treated samples indicates 1 - 2 additional peaks for the unknown compounds when compared to control samples.

Keywords : Andrographolide, Salicylic acid, Suspension culture.

INTRODUCTION

Plant cell culture is considered as a useful technique for the large scale production of plant secondary metabolites under *in vitro* level beside its utility for micropropagation. The production of these bioactive metabolites can be achieved by elicitation, cell immobilization, permeabilization (Brodelius, 1988, Mendhulkar *et al.* 2009), alteration in media composition etc.

Andrographis paniculata (Burn f.) Nees commonly known as 'Kalmegh' belongs to Acanthaceae and it is a well known medicinal plant. The plant is recommended for its drug utility in Indian Pharmacopoeia and widely used in Ayurveda, Unani, Siddha and Homeopathy systems of medicines.The plant is reported to possess terpenoids and flavonoids. The major terpenoids *viz.*, 14-deoxy andrographolide, andrographolide, neoandrographolide and 14-deoxy-11, 12-didehydro andrographolide are the active constituents in this plant system. The main active constituent is andrographolide which is reported to possess liver stimulant properties, astringent, anodyne, tonic and alexipharmic properties, and is useful in dysentery, cholera, diabetes, consumption, infulenza, bronchitis, swellings and itches, piles and gonorrhea (Zhao and Frang, 1991). The most significant pharmaceutical properties of this plant ar anticancerous (Kumar *et al.*, 2004) and anti-HIV (Calabrese *et al.*, 2000)

Salicylic acid has been successfully used inducing anthraquinone phytoalexin production in *Rubia cordifolia* (Bulgakov *et al.*, 2002), alkaloid production in *Brugmansia candida* (Pitta-Alvarez *et al.*, 2000), bilobalide and ginkgolides in cell cultures of *Ginkgo biloba* (Choi *et al.*, 2006).

In previous study (Butcher and Connolly, 1971), the synthesis of novel compounds *viz.*, paniculides A, B and C have been reported but the active constituent andrographolide was found to be absent in cultured cells of *Andrographis paniculata*.

In present study, an effort was made to trigger the synthesis of andrographolide in cell suspension of *A. paniculata* using an eliciting agent, salicylic acid.

MATERIAL AND METHODS

The seedlings of *Andrographis paniculata* were collected from Keshaw Shristi Nursery located at Bhayendar, Mumbai and grown in the pots in Institute of Science, Mumbai. Young leaves of *A. paniculata* were picked from mature plants grown in pot. The leaves were first washed under running tap water followed by Teepol for 1 min. The leaves were again washed with running tap water to remove Teepol. Later, the explants were surface sterilized with 0.1%$HgCl_2$ for 8 min. and rinsed with sterilized distilled water five times.

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The leaves were cut into 1-2 cm size leaf explants under aseptic condition and inoculated onto static MS medium supplemented with 2, 4-D and BAP (1.0 : 0.5). The pH of the medium was adjusted to 5.8 by NaOH. The matured callus was obtained after 10-12 weeks. The cultures were maintained at 25° ± 0.2°C under 16 hrs light condition.

The calli obtained were cut aseptically into small pieces and transferred to liquid MS medium to raise cell suspension using same medium composition execpt Agar. The cell suspenion culture was grown in gyratory shaker with 120 r.p.m. at 25 ± 1°C under dark condition for 16 days. The treatment of Salicylic acid was administered on day 15 with 0.05 mM, 0.5 mM and 1.5 mM concentrations for 24 hrs.

After 24 hrs treatment with salicylic acid, the cells were separated through sieve and kept in oven at 60°C for 24 drying. The cells were weighted for calculating dry weight. At same time leaves of *A.paniculata* were also dried in oven. The dried cells were powdered using mortar and pestle. 100 mg of each sample powder was extracted in 2 ml of methanol using ultrasonicator for 10 min. Standard andrographolide solution was prepared by dissolving 1 mg of andrographolide in 2 ml of methanol. The extract was centrifuged and supernatant was used for HPTLC analysis.

For HPTLC analysis, the samples were injected onto 10 x 5 TLC silica plate using automatic TLC sampler III. (CAMAG, Switzerland). Samples and reference standard were applied on TLC plate as 8 mm wide bands, positioned 20 mm from the bottom the distance between the two tracks was adjusted to 13.3 mm. After loading samples, the plate was put in Camag twin trough glass tank which was presaturated with mobile phase Chloroform: methanol (7 : 1) till the mobile phase travel up to 8 cm from bottom. After running mobile phase, the plate was dried using hair dryer and sprayed with spraying reagent (5 % H_2SO_4 in Methanol) and heated on hot plate at 100°C for 2 min. The plate was scanned at 230 nm in HPLC scanner (CAMAG TLC Scanner 3) for detection and quantification of andrographolide in samples.

RESULTS AND DISCUSSION

For induction of callus, 2,4-D along with Kinetin and BAP was used. The response of hormones on callus induction was studied (Table 1). Among the studied hormone combinations, 2,4-D:BAP was found most suitable for callus induction (Fig.1). The callus was obtained after 10-12 weeks. The surface sterilization of leaf explants with $HgCl_2$ was not good enough particularly in rainy seasons. However, this problem was overcome by treating the leaf explants with 0.5% Bavistin (an anti fungal) for 20-30min. 0.5% Bavistin was prepared by dissolving 0.5 gm of Bavistin powder in 100 ml D.W. The solution was autoclaved at 121°C for 15 min. before use.

HPTLC chromatogram of salicylic acid treated cell suspensions showed different bands for unknown compounds. For detection of andrographolide in salicylic acid treated samples, standard andrographolide was used as a reference compound (Fig. 3 & 4) which appeared at R_f value - 0.33. The standard andrographolide indicates a distinct band on HPTLC chromatogram (Fig. 2). When standard andrographolide

Table 1 : Response of callus induction

Sr.No	Hormones	Concentration mg/l	1st week	2nd week	3rd week	4th week
1.	2,4-D	0.5	-	≈	+	++
		1.0	-	≈	+	++
		2.0	-	+	++	•
		3.0	-	+	++	•
2.	2,4-D Kn	0.5:0.5	≈	+	++	++
		1.0:0.2	≈	+	++	++
		1.0:0.5	≈	+	++	++
		1.5:0.5	≈	+	++	++
		2.0:0.5	≈	+	++	++
3.	2,4-D: BAP	1.0:0.5	+	++	+++	++++
		1.5:0.5	+	++	++	+++
		2.0:0.5	+	++	+++	+++
		0.5:0.5	+	+	++	+++
4.	2,4-D : Kn+BAP	1.0:0.25+0.25	+	++	++	+++
		1.5:0.25+0.25	≈	+	++	++
		2.0:0.25+0.25	≈	+	++	++
		0.5:0.25+0.25	≈	+	++	++

- : no response, ≈ : callus initiation, • : browning of callus, +: growth of callus
++: growth of callus, +++: growth of callus, ++++: growth of callus.

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Fig 1. a. *Andrographis paniculata* Ness b. Callus initiation c. 12 week old mature callus d. Cell suspension

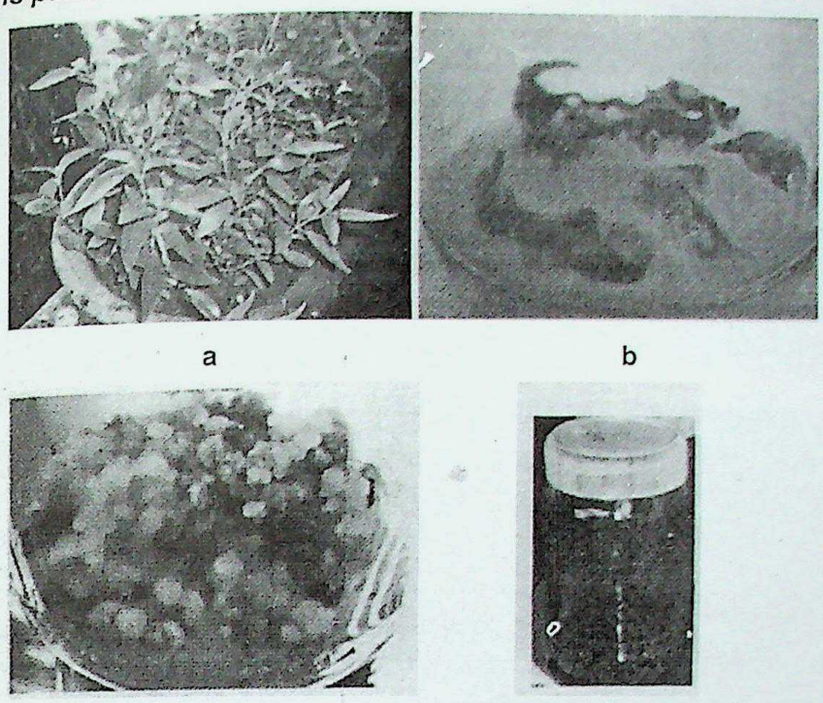
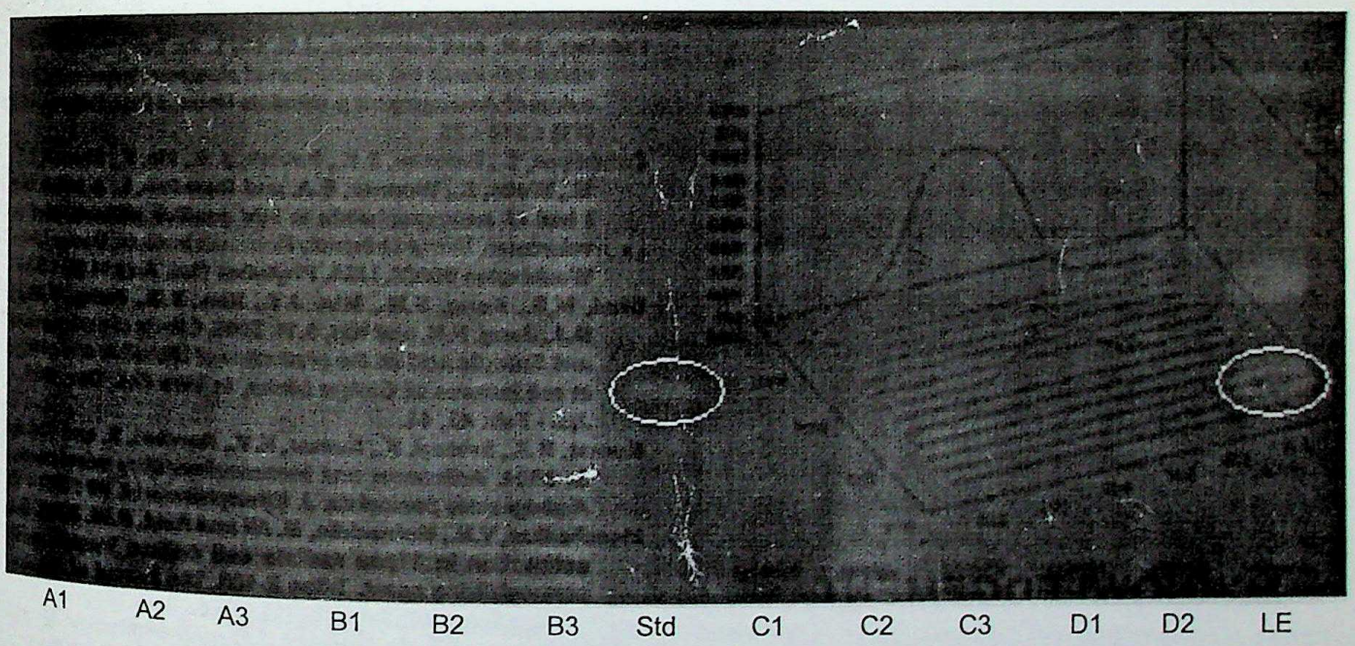


Fig 2. HPTLC Chromatogram

Image @ 366nm after Derivatization with 5% H_2SO_4 in MeOH



A : 0.05 mM Salicylic acid **B :** 0.5 mM Salicylic acid **C :** 1.5 mM Salicylic acid
D : Control **Std :** Andrographolide **LE :** Leaf extract

spectra was analyzed, it matched with leaf sample spectra revealing the presence of andrographolide in leaf but no andrographolide spectra was found in treated cell culture samples. However, extra peaks for new compounds in Salicylic acid treated samples were detected by HPTLC analysis compared to control. The factors have been investigated for the formation of paniculides A, B and C rather than andrographolide in cultured cells of *A. paniculata* (Butcher and Connolly, 1971). There is no direct link of medium composition, light and temperature with biosynthesis of andrographolide. Although the cells of *A. paniculata* are incapable of synthesizing andrographolide but the genes responsible for andrographolide might be in inactive form. The presence of some new peaks in cell culture samples treated with salicylic acid indicates the formation of known compounds. In Tobacco cultured cells (Taguchi *et al.*, 2001), salicylic acid triggers the expression of genes responsible for biosynthesis of secondary metabolites. Salicylic acid has been successfully used for inducing anthraquinon phytoalexin production in *Rubia cordifolia* (Bulgakov *et al.*, 2002), alkaloid production in *Brugmansia candida* (Pitta-Alvarez *et al.*, 2000). Other elicitors can be tried for biosynthesis of andrographolide at *in vitro* level.

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Fig 3. Peaks generated in cultured cells sample

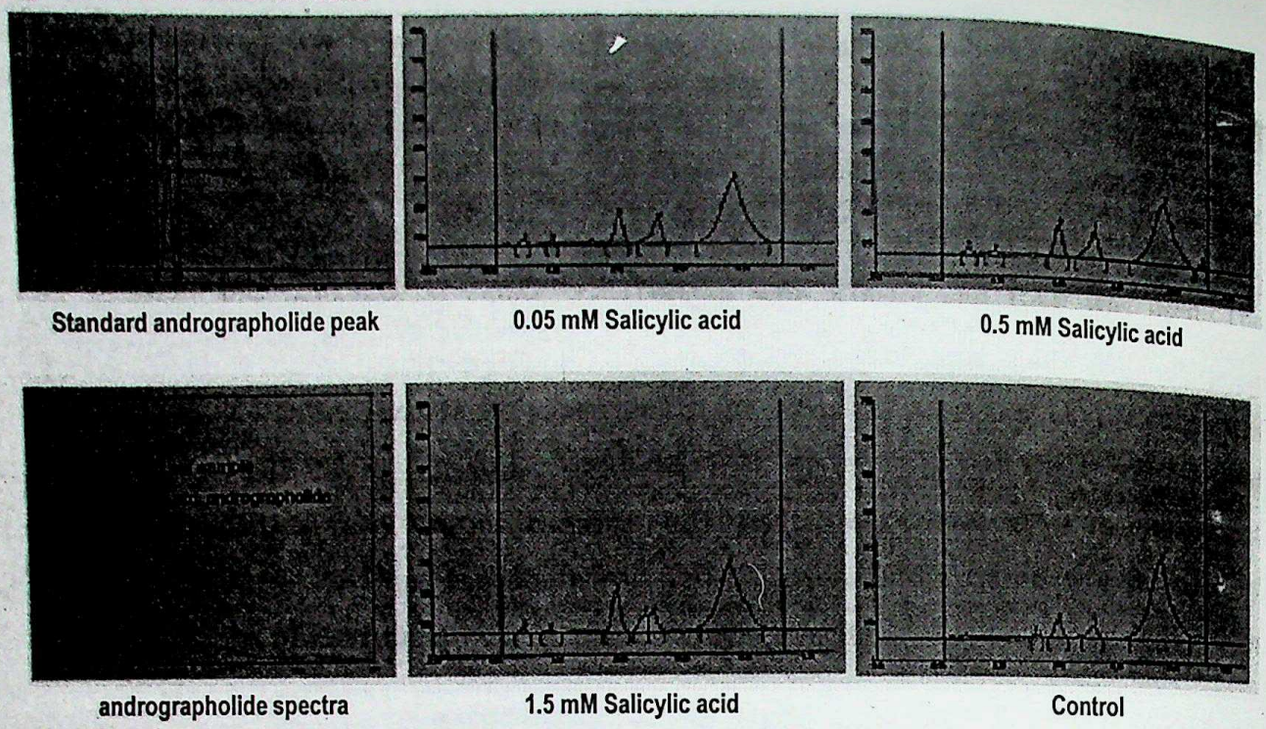
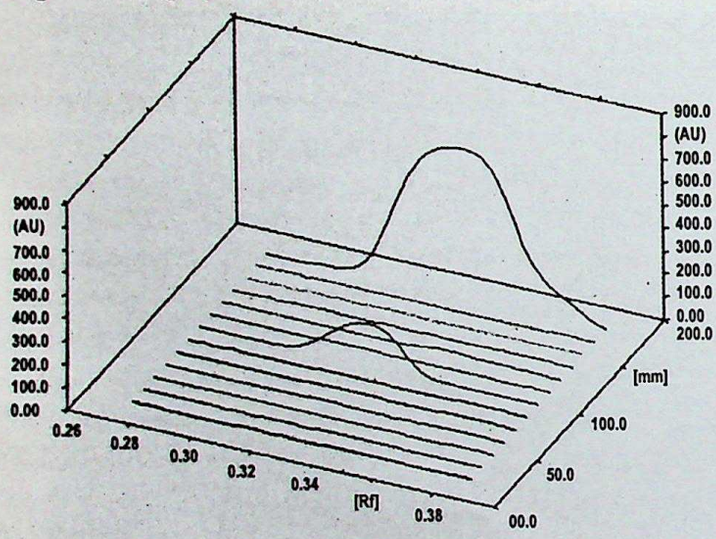


Fig 4. Andrographolide spectrum (All tracks at 230 nm)



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DNA Sugar Damage Induced by Different Oxidants and Effect of Aqueous and Alcoholic Extracts of *Malva sylvestris*

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Free radical induced damage to DNA can result in deleterious biological consequences such as the initiation and promotion of cancer. In the present study DNA damage inhibition was observed in Calf thymus DNA when treated with *Malva sylvestris* crude extracts. DNA was treated with oxidant complex (Ferric nitrate/ Ascorbic acid/H_2O_2) and 70% of DNA damage was observed. However in the presence of crude aqueous and alcoholic extracts of *Malva sylvestris,* DNA damage inhibition was observed in a dose dependent manner.

Keywords : DNA Damage, Calf thymus DNA, *Malva sylvestris*, Oxidant.

INTRODUCTION

Hydrogen peroxide in response to the indirect effects of ionizing radiations generates OH* radical that damages the DNA. In addition to thymine, the other DNA bases like cytosine and guanine are also subjected to the oxidizing effects of ionizing radiations (Dizdaroglu *et al.,* 1985). Ferric nitrate and ascorbic acid in the presence of different hydroperoxides causes oxidation of DNA bases (Kasai, 1997). Hydrogen peroxide can probably react with iron or copper ions to form hydroxyl radical, which is responsible for the oxidation of nitrogen bases and denaturation of DNA. Attack of OH* radical on the deoxyribose sugar produces a variety of different products some of which have been found mutagenic. Recently, much attention has been focused on the studies of plant flavonoids to protect DNA damage induced by different oxidants under *in vitro* conditions. Plant flavonoids a group of polyphenolic compounds that occur ubiquitously in plants have been demonstrated to protect DNA from oxidant-mediated damage under *in vitro* conditions, however flavonoids may also induce DNA damage, particularly at high concentrations (Johinson *et al.,* 2004).

Epidemiological studies strongly support the idea that increased consumption of flavonoids containing foods is associated with health maintenance and prevention of chronic and degenerative diseases (Rice *et al.*, 1996; Lemanska *et al.*, 2001). The antioxidant properties of flavonoids have attracted increased interest and consequently have been extensively investigated mainly in *in vitro* (Doulias *et al.,* 2005). Recently it has been found that the intracellular redox-active iron is a crucial mediator in H_2O_2 induced DNA damage (Duthie *et al.*, 1997). The crude extracts of many plants have been reported to modulate antioxidant enzyme levels, protect against inoizing radiation induced DNA damage, and protect damages to gastrointestinal, reproductive and central nervous system (Arora *et al.,* 2005). The present study was undertaken to investigate the protective effects of aqueous and alcoholic extracts of *Malva sylvestris* against DNA damages mediated by ferric nitrate, ascorbic acid and other peroxide oxidants.

MATERIAL AND METHODS

Plant material preparation : The plant material was collected from different locations of the Kashmir valley and authenticated by Dr. Irshad Ahmad Nawchoo (Department of Botany) and Akhter Hussain Malik (Curator, Centre for Plant Taxonomy, University of Kashmir). The plant material was shade dried and extraction was carried out by using the method of David *et al.*, 2000. Homogenize (10% W/V) dried plant material in water and alcohol and agitate it for 2 hours at 4°C, filtered and then centrifuged at 5000g for 10 minutes. The supernatant was dried in a rotary vacuum evaporator and the extract stroed at 4°C.

Preparation of DNA solution : A stock solution of DNA (2 mg/ml) was prepared by dissolving known weight of highly polymerized sodium salt of Calf thymus DNA in appropriate volume of Tris. HCl buffer, (0.001 M. pH 7.5), NaCl (0.01 M) and EDTA 2 x 10^{-4} M in salt free conditions. The solution was stored below 4°C for 48 hours before use.

DNA sugar damage assay : It was assayed by method of Gutteridge and Wilkinson (1983). The hydroxyl radical is generated by using ferric nitrate, ascorbic acid and hydrogen peroxide. The reaction mixture in a total volume of 2 ml contained 1 ml of Calf thymus DNA (1 mg/ml), 0.57 ml of Tris HCl buffer (0.001 M, pH 7.5), 0.2 ml of Fe^{3+} (20 mM), 0.2 ml of

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ascorbic acid (100 mM) and plant extracts were added wherever needed. The reaction mixture was incubated at 37°C for 24 hours in a water bath shaker. After incubation the deoxyribose degradation was assessed by estimating the formation of thiobarbituric acid reactive species (TBARS). 1 ml of 1.67% of thiobarbitutirc acid (TBA) was added in each tube and they were kept in a boiling water bath for 10 minutes. Tubes were cooled in an ice bath and centrifuged at 10000 g for 5 minutes. The TBARS was measured spectrophotometrically by taking absorbance at 532 nm. The results were expressed as percentage inhibition of deoxyribose oxidation, as determined by the following formula.

Percentage inhibition = [(A–B)/A] x 100

Where A was the malondialdehyde produced by Fenton reaction treated alone, and B was the malondialdehyde produced in the presence of *Malva sylvestries* extracts/known antioxidant.

RESULTS

Fig 1 shows the effect of different oxidants on deoxyribose sugar *in vitro*. Fe^{3+} / A.A in the presence of H_2O_2 was used to induce deoxyribose sugar damage in Calf thymus DNA following 24-hour incubation and the damage was assessed by measuring TBARS formation at absorbance 535 nm. Hydrogen peroxide alone did not induce appreciable deoxyribose sugar damage, but in the presence of Fe^{3+} / A.A. damage was increased many fold. Cumene hydroperoxide and tertiary butyl hydroperoxide alone or in the presence of Fe^{3+}/ A.A did not cause any significant increase in DNA damage. In

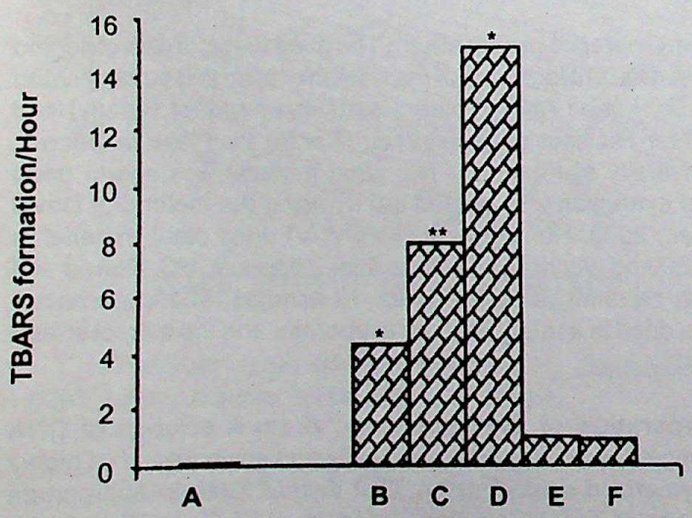

Fig. 1 . A represents DNA alone (control), **B** represents DNA + Ferric nitrate (20mM) and Ascorbic acid (500mM), **C** represents DNA and Hydrogen peroxide (30mM), **D** represents DNA + Ferric nitrate/ Ascorbic acid and Hydrogen peroxide, **E** represents DNA and Cumune hydroperoxide (5mM), **F** represents DNA tertiary butyl hydroperoxide (5mM). Each value represents mean ± S.E of six experiments
*** P < 0.001 with respect to their control.
** P < 0.01 with respect to their control.
* P < 0.05 with respect to their control.

Table 1 : Effect of *Malva sylvestris* extracts on free radical mediated DNA sugar damage.

Extracts	Inhibition (%)		
	1000µg/ml	100µg/ml	10µg/ml
Catchin (control)	94	75	55
Aqueous	20	14	08
Alcoholic	30	23	12

the presence of Fe^{3+} / A.A/ H_2O_2, 14.5 nmoles of TBARS were produced following 24-hour incubation.

The presence of various concentrations (10-1000 µg/ml) of *Malva sylvestris* extracts prevented the free radical mediated DNA sugar damage in a dose dependent manner (Table 1).

DISCUSSION

Hydroxy radical is a highly reactive radical formed in biological systems and capable of damaging almost every molecule found in living cells (Halliwell, 1991). This radical has the capacity to induce carcinogenesis, mutagenesis and rapidly initiates lipid peroxidation (Rajesh Manian *et al.*, 2008). *In vitro*, (OH*) were generated by a mixture of Fe^{3+} - H_2O_2 and ascorbic acid and were assessed by monitoring the degraded fragments of deoxyribose, through malonaldehyde (MDA) formation. If any plant extract or drug scavenges the hydroxyl radical, they may either scavenge the radical or may chelate the Fe^{2+} ion making them unavailable for the Fenton's reaction. Plant extracts containing perticularly polyphenols are reported to quench oxygen-derived free radicals by donating a hydrogen atom or an electron to the free radical (Mi-Yae Shon *et al.*, 2003) or neutralize free radicals by their chelating ability due to their high nucleophilic character of the aromatic ring (Becana & Klucas, 1992). In this study the extracts of *Malva sylvestris* were found to exhibit a dose dependent hydroxyl radical scavenging activity (Table 1). The radical scavenging property of *Malva sylvestris* as evidenced from the studies provides a platform for its future evaluation as a functional food. Concerted studies are needed to isolate and purify the active components from *Malva sylvestris* for its understanding and better utility.

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Effect of Fluoride on Catalase Activity in Two Varieties of Mulberry (*Morus alba*. L.)

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The activity of antioxidant enzyme, Catalase (CAT; EC 1.11.1.6) under fluoride toxicity was studied in mulberry leaf . Mulberry cultivars M-5 and V-1 differ in their response to fluoride, the latter being more sensitive to fluoride toxicity. Fluoride treatment reduced the activity of Catalase in fluoride stressed mulberry leaves . The activity of reduction in Catalase is time and dose dependent. Being a strong oxidizing agent, fluoride is capable of interacting with Catalase enzyme. The results obtained in the study clearly indicated the inhibitory effect of fluoride toxicity.

Keywords : Catalase, Fluoride toxicity, Mulberry varieties.

INTRODUCTION

A variety of environmental stress like extremes of temperature, drought, soil salinity, heavy metals, fluorine compounds are known to cause oxidative damage to plants either directly or indirectly by triggering an increased level of production of reactive oxygen species (Comblea *et al.*, 1998). These reactive oxygen species include super oxide radical (CO_2^{-1}), hydroxyl radical (OH^{-1}) and hydrogen peroxide (H_2O_2) and products during membrane linked electron transport activities as well as by a number of metabolic pathways and in turn cause damage to the biomolecules such as membrane lipids, proteins, lipids ,chloroplast pigments, enzymes and nucleic acids (Mishra and Singhal, 1992). Plants have antioxident defence mechanism comprising of enzyme catalased.(Ali *et al.* , 2005). The catalases are enzymes which cause the evolution of oxygen on attacking the substrate hydrogen peroxide generation during the photo-respiration and β-Oxidation of fatty acids (Sharma and Davis , 1977). The reactive species of oxygen have the potential to cause considerable damage to chloroplasts, other membrane systems and cellular macromolecules. Hydroxyl radicals, in particular are so reactive; they can attack and damage almost every molecule of living cells (Cadena, 1989). The prolonged presence of these active oxygen species is not compatible with cell function or structure. The main cellular components susceptible to damage by free radicals of oxygen are lipids (Per oxidation of unsaturated fatty acids in membranes), proteins (denaturation of enzymes), carbohydrates (cleavage of polysaccharides) and nucleic acids (scission of DNA strands *i.e.*, mutation) (Monk *et al.*, 1989). As fluoride is one of the toxic substance present in both aquatic as well as terrestrial environments and mulberry crop serves as chief feed for silkworms, the present study was undertaken to determine the fluoride induced possible alteration in the oxidative damage to mulberry and likely alterations in behavior of the enzyme catalase of antioxidant defense system in mulberry leaves.

MATERIAL AND METHODS

The mulberry leaves of varieties V_1 and M_5 for the present study were procured from the Regional Sericultural Research Station, Anantapur, Andhra Pradesh, India. The experiment was conducted in 45 days old leaves. They were detached from the plants and washed in deionized water and were surface sterilized with 0.1 per cent mercuric chloride solution for 30 seconds, then washed with distilled water.

The leaf bits were placed in Petri dishes of 20 cm diameter, containing distilled water or fluoride solutions. Four leaf bits were kept in each Petri dish. Fluoride is available in many salts forms like, sodium fluoride, calcium fluoride, aluminum fluoride etc. Sodium fluoride with molecular formula weight of 41.988, is used in the investigation to study the effects of fluoride on mulberry leaf. Different concentrations of the above salt *i.e.*, 100ppm, 200ppm and 300ppm were prepared in distilled water and distilled water was alone served as control. Three Petri dishes were placed for each concentration of fluoride. Petri dishes were kept under a light intensity of approximately 150 wm^{-2} and temperature of 27° ± 3°C . The solutions were replaced every day with fresh ones. The samples were estimated after 2 days (48hrs), 4 days (96 hrs), 6 days (144 hrs) and 8 days (192 hrs) of incubation for physiological and biochemical studies. The leaf material was homogenized in chilled phosphate buffer (pH-7). The homogenate was filtered and used for assaying catalase. Catalase activity was assayed by the method of Barber (1980). The reaction mixture contained enzyme extract, hydrogen

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Table 1 : Catalase (units gm^{-1} fresh wt h^{-1}) activity in control and on exposure to different concentrations of fluoride at 2nd, 4th, 6th and 8th days in the leaves of mulberry varieties .

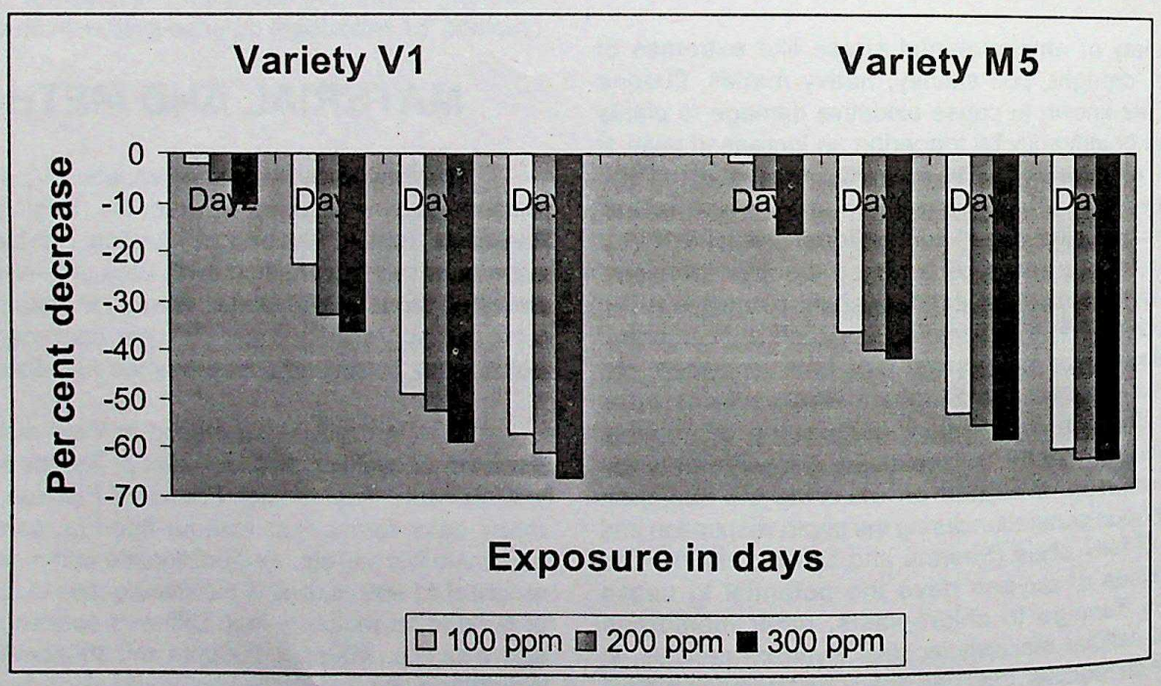
	V-1 Variety				M-5 Variety			
Day	Control	100ppm	200ppm	300ppm	Control	100ppm	200ppm	300ppm
2	1.352[a]	1.323[b] (-2.114)	1.276[c] (-5.621)	1.213[d] (-10.281)	1.213[a]	1.193[b] (-1.648)	1.127[c] (-7.089)	1.016[d] (-16.240)
4	1.512[a]	1.174[b] (-22.354)	1.018[c] (-32.671)	0.965[d] (-36.177)	1.521[a]	0.974[b] (-35.963)	0.912[c] (-40.039)	0.889[d] (-41.551)
6	1.610[a]	0.823[b] (-48.88)	0.772[c] (-52.049)	0.665[d] (-58.695)	1.691[a]	0.795[b] (-52.986)	0.753[c] (-51.470)	0.701[d] (-58.545)
8	1.819[a]	0.788[b] (-56.679)	0.717[c] (-60.582)	0.623[d] (-65.674)	1.736[a]	0.677[b] (-61.002)	0.642[c] (-63.018)	0.623[d] (-63.594)

*Each value is a mean of eight estimations

** Per cent decrease / increase over control is given in parenthesis.

*** Means within a row followed by the same letter are not significantly different (P>0.05) from each other according to Duncan's multiple range tests.

Figure 1: Per cent decrease over control in the Catalase activity in the leaves of different mulberry varieties on exposure to different concentrations of fluoride at 2, 4, 6 and 8 days.



peroxide and phosphate buffer (pH - 7). The reaction was stopped by adding conc. sulphuric acid and the residual hydrogen peroxide with Potassium Permanganate.

Statistical analysis : The data represents the mean values of 8 replicates. The data were analyzed by Duncan's Multiple Range Test (Duncan, 1955). The differences were considered significant if P was < 0.05. The mean values were compared and lower case letters used in the table to highlight the significant differences between the treatments.

RESULTS AND DISCUSSION

Enzymic antioxidative defense system includes catalase. With the increasing levels of fluoride treatment a concomitant decline in catalase activity was observed in leaves of mulberry at all exposure periods (Table 1 and Fig.1). Compared to M_5 mulberry variety, a greater inhibition in catalase activity was observed in V_1. The results showed a decrease in the level of catalase activity indicating that fluoride inhibits catalase activity. Further, results are in conformity with the observations of many other investigators who have also found that fluoride inhibited the catalase activity (Beers, 1955,

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Newman, 1962, Ali *et al.*, 2005, Cho and Seo , 2005) and concluded that iron atoms of the catalase have hydroxyl groups that might be replaced by low molecular weight anions, in sufficient concentrations, such that catalase is correspondingly inhibited. However, the fluoride *in vivo* may be causing a reduction in the amount of catalase and not just a simple inhibition of the catalase system as measured in the homogenates. Further, so far no evidence of stimulation of catalase is available in plants on exposure to fluoride. The finding further indicated the ability of M-5 for resistance of fluoride as it is indigenous to fluoride bearing soils of Anantapur region. Whereas the V-1 has not that much resistance to fluoride toxicity as it is a newly evolved variety. The degree of inhibition of Catalase activity is time and dose dependent.

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Colorimetric Determination of Cell Growth Phase and Detection of Andrographolide in Suspension Culture of *Andrographis paniculata* (Burm f.) Nees

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The present piece of work was carried out to study the cell growth phase and to detect the presence of andrographolide in cell suspension culture of *Andrographis paniculata* Nees. as a part of study related to secondary metabolic production under *in vitro* condition. The cell suspension was raised from callus induced in MS medium supplemented with 2, 4-D: BAP (1.0:0.5). The cell suspension was grown in Erlenmeyer flask for 30 days in gyratory shaker with rpm 110. The optical density was measured at 430-480 nm till stationary phase obtained. The detection of andrographolide in cultivated plant and in cultured cells of *A. paniculata* was done by HPTLC analysis. The standard andrographolide compound was scanned at 223 nm with Rf-value 0.41. The results obtained by analyzing the compounds in the cells grown under *in vitro* condition (30 days old cultured cells grown in MS medium) did not reveal the corresponding peak for standard andrographolide. It indicates either the cultured cells did not attend the productive stage within the period provided for growth or there may be metabolic interference in the synthesis of compound under *in vitro* condition. However, the sample analyzed from control plant indicated the presence of andrographolide.

Keywords : Andrographolide, Cell growth phase, Optical density.

INTRODUCTION

Andrographis paniculata Nees. Family- Acanthaceae is well known medicinal plant commoly known as Kalmegh. The plant is recommended for its drug utility in Indian Pharmacopoeia and widely used in Ayurveda, Unani, Siddha and Homeopathy systems of medicines. The herb is reported to possess liver stimulant properties, astringent, anodyne and tonic properties and is useful in dysentery, cholera, diabetes, influenza, bronchitis, swellings, itches, piles and gonorrhea (Zhao *et al.*, 1991), anticancerous (Kumar *et al.*, 2004) and anti-HIV (Calabrese *et al.*, 2000). A decoction of the plant is a blood-purifier. It is also used as a cure for torpid liver, jaundice and dyspepsia (Trivedi *et al.*, 2001). The juice of macerated leaves and roots is used as tonic, febrifuge, stomachic, cholagogus and anthelmintic, stimulant (Warrier *et al.*, 1996).

The present work deals with the determination of cell growth phase in *Andrographis paniculata* Nees. in cell suspension by Colorimetric method and detection of andrographolide in cultured cells of *A. paniculata* as a part of study related to its bioactive compounds by *in vitro* technique.

MATERIAL AND METHODS

Leaf explants of *Andrographis paniculata* Nees. were surface sterilized with 0.1% (w/v) $HgCl_2$ solution for 8-10 min. Leaf explants inoculated on MS medium supplemented with 2,4-D:BAP (1.0:0.5). Callus was cultured for 8 weeks. Cell suspension was prepared from callus and cultured for 30 days under gyratory shaker (110 rpm) in dark. (Fig. 1 a,b,c) Optical Density (OD) was measure daily for 30 days at 430-480 nm using Colorimeter.

After 30 days of culture, the cells were separated by sieve and dried in oven at 60ºC for 24 hrs. The dried cells were powdered using mortar and pestle. At same time leaves of *A. paniculata* were also dried in oven. The dried cells were powdered in mortar and pestle. 100 mg of each sample powder was extracted in 2 ml of methanol using ultrasonicator for 10 min. Standard andrographolide solution was prepared by dissolving 1 mg of andrographolide in 2 ml of methanol. The extract was centrifuged and supernatant was used for HPTLC analysis.

For HPTLC analysis, the samples were injected onto 10 x 5 TLC silica plate using automatic TLC sampler III. (CAMAG, Switzerland). Samples and reference standard were applied on TLC plate as 8 mm wide bands, positioned 20 mm from the bottom the distance between the two tracks was adjusted to 13.3 mm. After loading samples, the plate was put in Camag twin trough glass tank which was presaturated with mobile phase Chlorofrom: methanol (7: 1) till the mobile phase travel up to 8 cm from bottom. After running mobile phase, the plate was dried using hair dryer and sprayed with spraying

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Table 1 : Optical Density of Cell Suspension.

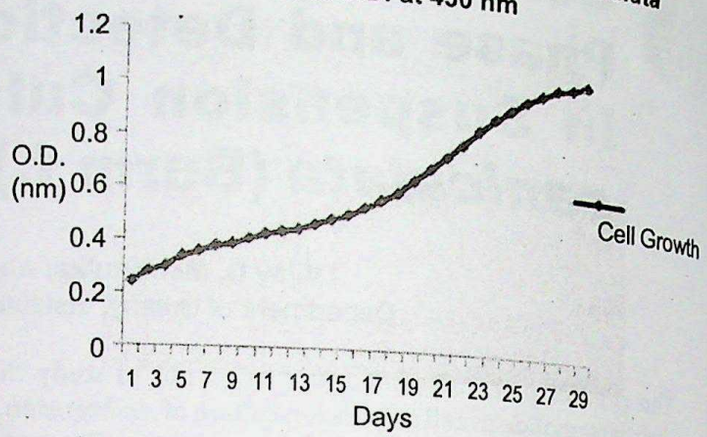
Day	430nm	480nm
2	0.24	0.25
3	0.28	0.28
4	0.30	0.32
5	0.34	0.37
6	0.36	0.40
7	0.38	0.43
8	0.39	0.46
9	0.41	0.47
10	0.43	0.48
11	0.44	0.49
12	0.45	0.51
13	0.47	0.55
14	0.49	0.59
15	0.51	0.63
16	0.54	0.66
17	0.57	0.71
18	0.60	0.75
19	0.65	0.80
20	0.70	0.84
21	0.75	0.90
22	0.80	0.96
23	0.86	1.02
24	0.91	1.09
25	0.95	1.15
26	0.99	1.20
27	1.02	1.25
28	1.05	1.28
29	1.06	1.29
30	1.08	1.30

reagent (5%H_2SO_4 in Methanol) and heated on hot plate at 100ºC for 2 min. The plate was scanned at 230 nm in HPLC scanner (CAMAG TLC Scanner 3) for detection and quantification of andrographolide in samples. The cell growth phase of *A. paniculata* Nees. was determined by colorimetric method for 30 days. The other methods of cell growth measurement like cell counting by hemocytometer, Packed Cell Volume (PCV) and dry weight basis causes cell demolition. The present study showed that Colorimetric method is useful for determining the Plant cell Growth phase. Colorimetric method reduces the risk of contamination.

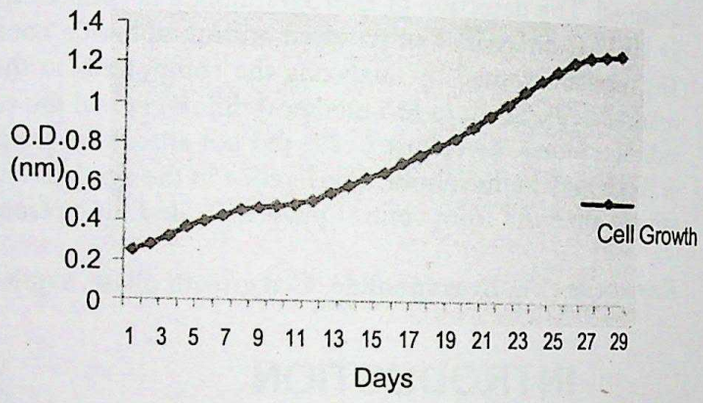
RESULTS AND DISCUSSION

The cell density was measured at 430-480 nm. The lag phase was measured between 3-9 d, exponential phase between 9-25d and stationary phase 25-30d (Table 1). The readings were recorded as an average of three separate flasks. Sung Z.R. (1975) have reported the colorimetric method which determine the plant cell growth. The Growth phases obtained can be used for eliciting the suspension culture. In HPTLC

Cell Growth Phase of *Andrographis paniculata* Nees. O.D. at 430 nm



Cell Growth Phase of *Andrographis paniculata* Nees. O.D. at 480 nm



analysis, the standard andrographolide compound was scanned at 230 nm with Rf-value 0.41 (Fig.2). The results obtained by analyzing the compounds in the cells grown under *in vitro* condition (30 days old cultured cells grown in MS medium) did not reveal the corresponding peak for standard andrographolide. The cultured cells have been found to produce novel compounds which are not found in intact plant. For example, Epchrosine from *Ochrosia elliptica* (Pawelka *et al.*, 1986). Pericine from *Picralima nitida* (Arens *et al.*, 1982), Rutacultin from *Ruta graveolens* (Steck *et al.*, 1971). It indicates either the cultured cells did not attend the productive stage within the period provided for their growth or there may be metabolic interference in the synthesis of compound under *in vitro* condition. However, the sample analyzed from control plant indicated the presence of andrographolide.

The studies on enhancement of bioactive compounds of medicinal plants by *in vitro* techniques have gained an importance since 1970 due to the promising results. Alkaloids and steroids are the largest compounds of interests to the researchers. However, some plants may respond some may not under such condition. The modification in media composition, hormonal combination and change in the protocol modules as per the suitability of experimental plant system are the key aspects required to be studied. Elicitation (Bulgakov *et al.*, 2002, Pitta-Alvarez *et al.*, 2000), cell permeabilization (Brodelius, 1988, Mendhulkar *et al.*, 2009) cell immobilization

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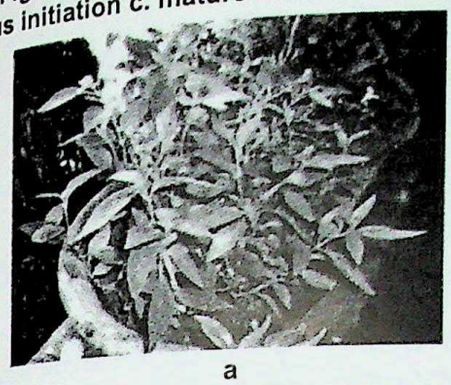
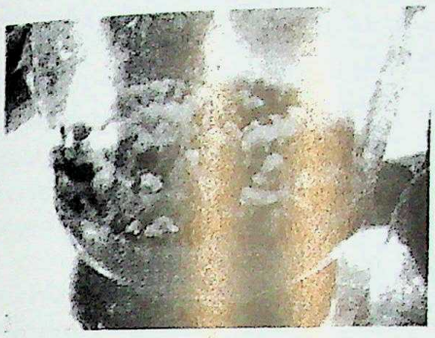
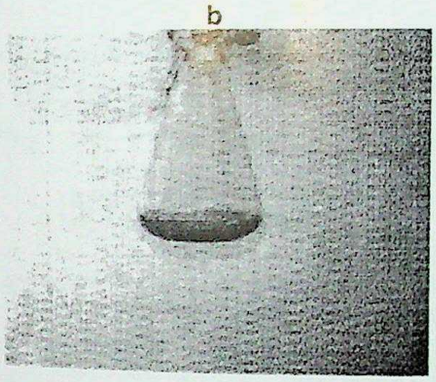
Fig. 1. a. *Andrographis paniculata* Ness b. Callus initiation c. mature callus d. Cell suspension



a



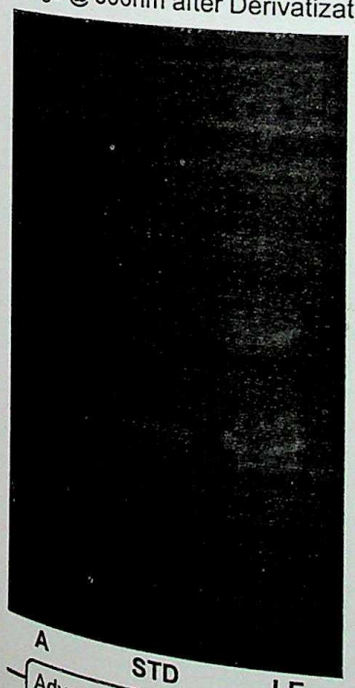
b



c

Fig. 2. HPTLC Chromatogram

Image @ 366nm after Derivatization with 5% H_2SO_4 in MeOH



A : Cell Suspension

STD: Standard andrographolide

LE : Leaf Extract

(Brodelius *et al.*, 1979, Lindsey *et al.*, 1983) are extremely helpful techniques that contribute to the approach of quantitative and qualitative improvement of metabolic prodcuts. The detection of andrographolide was carried out by HPTLC analysis. HPTLC analysis showed the presence of andrographolide only in leaf extract whereas no peak for andrographolide was found in cultured cells.

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In Vitro Assessment for Rhizogenesis from Nodal Explant, in *Centella asiatica* (Lin.) Urban

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Centella asiatica (Lin.) Urban. syn. *Hydrocotyle asiatica* (Brahmi synonymously known as Mandookaparni or Indian pennywort) was assessed for organogenesis particularly the rhizogenetic processes. Optimal results were obtained in half strength MS salt supplemented with varying concentration and combination of auxins (2, 4-D, NAA and IBA) and cytokinins (BAP and KN). Initiation of root from the callus and the explant were indicative of a number of physiological anomalies such as ageo-, diageotropic besides normal (+) - geotropism, with concomitant induction of callus like growth (CLG), originating possibly from the epidermis and epiblema. Maximum CLG was recorded in half MS + 2.5 mg l^{-1} NAA + 0.25 mg l^{-1} BAP after 4 weeks of culture duration and was evenly and sparingly distributed both in the primary roots in the secondary roots.

Keywords : *Centella asiatica*, Brahmi, Rhizogenesis, Nodal Explant, Physiological Tropic Anomalies, Callus Like Growth (CLG), 2, 4-D, IBA, BAP. KN.

Abbreviations : MS, Murashige and Skoog medium; CLG, Callus Like Growth; 2, 4-D, 2, 4-dichlorophenoxy acetic acid; IBA, indole-3-butyric acid; NAA, α - naphthalene acetic acid; BAP, 6-benzyl aminopurine; KN, kinetin.

INTRODUCTION

Complementary and Alternative Medicines System (CAMS) in past few decades have gained renewed interest that provide relief and panacea to multitude of people for these are safe, economical and powerful alternative cure to number of ailments, with limited treatment options in the modern medicines (Wojcikowski, David, Glenda, 2006) particularly those pertaining to Chronic Kidney Disease (CKD).

Centella asiatica (Lin.) Urban. syn. *Hydrocotyle asiatica* (Brahmi synonymously known as Mandookaparni or Indian pennywort) family Apiaceae, an important medicinal plant, with applications in allopathic, ayurvedic, unani, siddha and horopathic (tribal medicine) systems of medicines. In Jharkhand State particularly, and the adjoining areas (Bihar, West Bengal, Orissa and Chhattisgarh) the plant is very popular among the tribal and the general populace, consumed as pot-herbs (saag) and sold in the village weekly bazaar under the popular name 'Beng Saag'. It has curative properties in the treatment of vast and diverse types of human diseases related to hepatic ailments like steatosis, hydropic degeneration, necrosis and renal dysfunction (Antony *et al.*, 2006), and also neurological problems such as epilepsy, stroke, multiple sclerosis and other neuropsychiatry problems (Barbosa, Pittella, Gattaz, 2008) and dermatological complications besides its strong and efficacious well documented antioxidant property (Jayashree, Kurup Muraleedhara, Sudarslal, Jacob, 2003). The therapeutic values have been attributed to the presence of bioactive terpene acids; asiatic acid, madecassic acid and their respective glycosides, asiaticoside and madecassoside and triterpene like centellin, asiaticin, and centellicin (Siddiqui *et al*, 2007). with proven healing potentiality in various animal models. Studies of micropagation of *Centella asiatica* is well documented (Sivakumar, Alagumanian and Rao, 2006). Unlike, the commendable work done on the phytochemistry and therapeutic properties on different animal models, the micropropagation in *Centella asiatica* is scanty and inadequate (Tiwari, Sharma, Tiwari, Singh, 2000). The present communication organogenesis (callus, embryogenesis) with particular reference to rhizogenesis (root initiation, nature of tropic movements) is highlighted and discussed.

MATERIAL AND METHODS

Centella asiatica (Lin.) Urban. (Brahmi), collected from the paddy fields (native conditions) during Feb -Apr. 2007 were grown in earthen pots in kitchen garden. Freshly harvested plants were used for the experimental purposes. The stem runners were removed of all the leaves and roots and cut between two consecutive internodes to remove nodes. The explants were washed in running tap water followed by further rinsing in distilled water and surface sterilized in 50 % v/v ethanol for 30 seconds and agitated vigorously in 0.1 % w/v mercuric chloride for 10 min. The explants were transferred

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Table 1 : Synergistic effect of different combination and concentration auxins (NAA, IBA, 2, 4-D) and cytokinins (KN, BAP) in half salt strength MS on the induction of callus embryo and rhizogenesis in *Centella asiatica*. (C = Compact, F = Friable, AG = Ageotropic, DG = Diageotropic, (+) = Positive Geotropic).

Combination & Concentration of Auxins + Cytokinins (in mg l^{-1})	Callus		Embryo		Rhizogenetic Process		
	Days taken for callusing	Nature	Days taken for E- genesis	%	Days taken	% of roots with CLG	Nature of roots
IBA + KN							
1.0 -	-	-	-	-	-	-	-
1.5 -	-	-	-	-	-	-	-
2.0 0.20	33 ± 7	C	41 ± 6	3	45 ± 4	0.0	AG
2.5 0.25	28 ± 3	C	48 ± 8	2	51 ± 6	0.0	AG
3.0 0.30	30 ± 6	C	37 ± 6	4	43 ± 4	0.0	AG
NAA + KN							
1.0 -	-	-	-	-	-	-	-
1.5 -	-	-	-	-	-	-	-
2.0 0.20	23 ± 5	C	28 ± 3	7	32 ± 2	0.0	(+)
2.5 0.25	21 ± 3	C	25 ± 4	8	31 ± 2	0.0	(+)
3.0 0.30	24 ± 4	C	24 ± 3	8	32 ± 2	0.0	(+)
2, 4-D + KN							
1.0 -	-	-	-	-	-	-	-
1.5 -	-	-	-	-	-	-	-
2.0 0.20	21 ± 3	F	24 ± 3	20	21 ± 2	0.0	(+)
2.5 0.25	22 ± 2	F	24 ± 3	22	22 ± 2	0.0	(+)
3.0 0.30	22 ± 2	F	24 ± 3	22	22 ± 2	0.0	(+)
IBA + BAP							
1.0 -	-	-	-	-	-	-	-
1.5 -	-	-	-	-	-	-	-
2.0 0.20	28 ± 5	C	48 ± 6	2	51 ± 6	0.0	(+)
2.5 0.25	33 ± 4	C	43 ± 5	3	53 ± 4	0.0	(+), DG
3.0 0.30	30 ± 5	C	41 ± 5	3	53 ± 4	0.0	(+), DG
NAA + BAP							
1.0 -	-	-	-	-	-	-	-
1.5 -	-	-	-	-	-	-	-
2.0 0.20	23 ± 5	C	28 ± 3	7	22 ± 2	10.0	(+)
2.5 0.25	21 ± 3	C	25 ± 4	8	21 ± 2	12.0	(+)
3.0 0.30	24 ± 4	C	24 ± 3	8	22 ± 2	12.0	(+)
2, 4-D + BAP							
1.0 -	-	-	-	-	-	-	-
1.5 -	-	-	-	-	-	-	-
2.0 0.20	23 ± 2	F	24 ± 3	20	21 ± 2	27.0	(+)
2.5 0.25	22 ± 3	F	24 ± 3	22	22 ± 2	32.0	(+)
3.0 0.30	22 ± 3	F	24 ± 3	22	22 ± 2	30.0	(+)

half salt strength MS media fortified with growth regulators auxins (2, 4-D, NAA and IBA) and cytokinins (BAP and KN) in different combinations (IBA + KN, NAA + Kn, 2, 4-D + KN, IBA, + BAP, NAA + BAP, 2,4-D + BAP) and concentration (auxins in the concentration ranging between 1.0 to 3.0 and cytokinins between 0.20 to 0.30) (Table 1). Lower concentration of auxins (1.0 mgl^{-1} to 1.5 mgl^{-1}) without CK was compared *vis-a-vis* higher auxin concentration (2.0 mgl^{-1} to 3.0 mgl^{-1}) in different combinations of CK's (KN and BAP) and concentration (0.20 to 0.30 mgl^{-1}) range. The culture were sub-cultured and transferred to freshly prepared media after every 20 to 25 days. The pH of the medium was adjusted at 5.8 (1 N NaOH or/and 1 N HCl) and the incubation temperature of 23 ± 2 °C under fluorescent lighted condition in culture room. The data pertaining to induction of callus (period of incubation and nature of the callus (Friable/Compact), the embryogenesis (time taken and frequency explant $^{-1}$) and rhizogenesis (time taken, frequency of roots) with CLG and the tropic nature of the roots were recorded and scored. Each treatment was replicated thrice to ascertain the repeatability and authenticity of the results.

RESULTS AND DISCUSSION

The nodal explants showed initiation of growth activity after minimum of three weeks of incubation (Table 1) with the proliferation of some friable compact calli which was depended on auxin and cytokinin combination and concentration. The optimum calli induction was recorded in 2.0 mg l^{-1} of 2, 4-D

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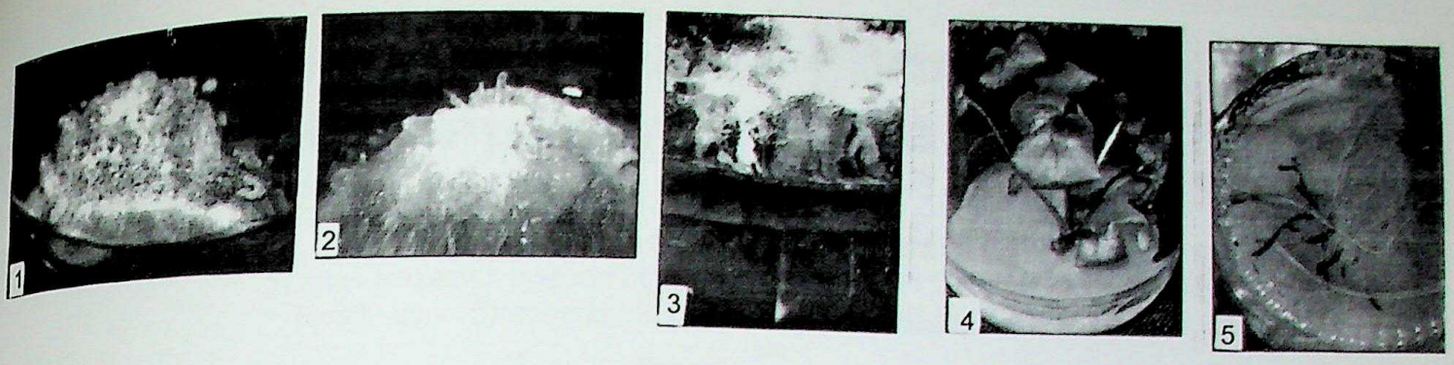

Legends:
Fig. 1: Friable Callus (after three weeks of inoculation) in HSMS + 2.5 mg l^{-1} 2, 4-D + 0.25 mg l^{-1} KN.
Fig. 2: Friable (more than three to four weeks old), in HSMS + 2.5 mg l^{-1} NAA + 0.25 mg l^{-1} BAP
Fig. 3: Growth of callus (more than four weeks old) in HSMS + 2.5 mg l^{-1} 2, 4-D + 0.25 mg l^{-1} BAP
Fig. 4: Growth of shoot and roots from explant (four weeks old) HSMS + 2.5 mg l^{-1} IBA + 0.30 BAP.
Fig. 5: CLG's over root surface (more than four weeks old) in HSMS + 2.5 mg l^{-1} of NAA + 0.25 mg l^{-1} of BAP

followed by NAA and IBA in combination with 0.20 mgl^{-1} of KN or BAP. However, the calli (Fig. 1) immediately after (3 - 5 days) attaining the maximal calli growth, some slender, erect shoot like or prostrate and filiform root like growth were recorded, which were either ageotropic (AG) or diageotropic (DG) (Fig. 2). However, by the onset of the fourth and the fifth week (media fortified with the combination of 2.5 mgl^{-1} of both 2,4-D or NAA and 0.25 to 0.30 mgl^{-1} of KN and BAP displayed profuse shoot and root growth with more less similar effects (Fig 2, 3 and 4). The maximal shoot generation was recorded in HSMS + 2.5 mgl^{-1} of NAA + 0.25 mgl^{-1} BAP with compact calli and rhizogenesis (growth of roots) was signified by the deeper penetration of the primary roots into the culture media followed by the emergence of secondary and tertiary roots, similar to the findings of host of other workers in *Centella* (Mohapatra, Barik and Rath, 2008) and various other plant materials (Madhavilatha and Singh, 2008). The prolonged culture period (more than four weeks) resulted into the formation of callus like growth (CLG) over the surface of the root which was white that turned brown later on. These CLG were either contiguous or intermittently distributed throughout the length of the root surface. The anatomical part or the tissues involved in the induction of the CLG could not be ascertained, however, it appeared that these were either from the epidermal (in case of primary roots) or the epiblema of the secondary roots. Such growth over the surface of the root cannot be attributed to any bacterial or ectotrophic mycorrhizal growth (major contaminant (fungal nature) usually present in cultures of *Centella* plants) (Joshee, Biswas and Yadav, 2007). Such growths were not recorded in other combinations of growth regulators, rules out the possibility of contaminant (mycorrhizal or bacteria) growth. It appeared that these CLG's were in integral part of the explant tissue, attibutable to the fact that such tissues are produced under specific physiological conditions that supplement the functions of root hairs or root cap (calyptra) or serving any other specific function.

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Early Yield and Growth Performance of Wild *Hevea* Germplasm in India

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Natural rubber with its center of origin in the Amazon rain forests of Brazil is a strategic industrial crop cultivated mainly in the South East Asian Countries. In the present study, a set of 545 wild *Hevea* accessions from three provenances of Brazil *viz.,* Acre (AC), Rondonia (RO) and Mato Grosso (MT), along with four popularly cultivated clones, were evaluated for their early yield growth performance in the first three years of growth in a nursery in the traditional rubber growing region of Kerala state, India. The characters studied include yield and growth characters such as girth, girth increment, plant height, crotch height and single leaf area. Highly significant clonal variability was observed for yield and all the growth characters *viz,* girth, girth increment, plant height and crotch height execpt single leaf area. The yield ranged from 0.3 g/t/t. (RO 2153) to 4.8 g/t/t. (AC 4394); girth ranged from 8 cm (AC 3559) to 19.4 cm (MT 200); girth increment from 1.1 cm (MT 2229, RO 5430) to 7.4 cm (RO 2846); single leaf area from 33.7 cm² (RO 2790) to 232.5 cm² (AC 3755); plant height from 2.4 m (AC 3559) to 7.5 m (7/118) and crotch height ranged from 1.7 m (AC 3559) to 5.5 m (AC 4114). The accessions were ranked using all the above parameters, execpt single leaf area, for overall performance, Rank sum values ranged from 38 to 2660 with a general mean of 1339. Correlations worked out among the six quantitative traits revealed that yield is significantly correlated with all the growth characters except single leaf area. Based on this study, the top 10% of the potential accessions showing early growth vigour and yield were identified which could be of use in future crop improvement programmes.

Keywords : *Hevea brasiliensis,* Wild germplasm, Genetic variation, Correlations, Rank sum.

INTRODUCTION

Natural rubber (*Hevea brasiliensis*) is a popularly cultivated species for its valuable raw material-rubber which is used in a number of rubber products in the world. Wickham collection comprised of a very small gene pool, since it was collected from a limited area of native Amazon region, Brazil (Schutles, 1977). Due to the human interventions, genetic resources of *Hevea* are fast depleting in the primary center of origin. The intensive directional selection over the years for yield alone has further narrowed the genetic base (Wycherley, 1969), and has further resulted in a slow down in genetic advances in recent breeding phases (Tan, 1981; Seguin *et al.,* 1995; Simmonds, 1989). The urgency of *Hevea* conservation was felt by the natural rubber industry, thus the International Rubber Research and Development Board (IRRDB) has organized an expedition during 1981 in the primary centre of origin of the crop, the Amazon basin, covering wide agro-climatic areas in the states, Acre (AC), Mato Grosso (MT) and Rondonia (RO) in Brazil, for collection and conservation of variability in the with *Hevea* germplasm. This resulted in collection of over 60,000 seeds and budwood from 194 exceptionally good trees (Ong *et al.,* 1983). These accessions were distributed to IRRDB member countries, and those received in India, are being conserved in conservation cum source bush nurseries.

As part of evaluation of this germplasm, data on various growth and juvenile yield were recorded in the early growth phase. The present study was undertaken to ascertain the extent of genetic variability in the population and identify potential accessions for using in the breeding programmes.

MATERIAL AND METHODS

The study was conducted at the Central Experiment Station of the Rubber Research Institute of India, Chethackal, Kerala state, India. A nursery was laid out in an augmented random block design during 2003, to characterize and evaluate the *Hevea* germplasm for various characteristics. A total of five hundred and forty five wild accessions from Acre (223), Mato Grosso (86) and Rondonia (229) provenances, seven other types along with four popular clones *viz.*, RRII 105, RRII 208, RRIM 600, PB 235 were included in this study. The spacing adopted was 1 x 1 m with five plants per plot. Provenance wise distributions of the accessions is given in Table 1. The recommended cultural practices of Rubber Board were followed.

The variability in yield (g/t/t) amd growth characters *viz.*, girth (cm) of the stem at a height of 30 cm above the bud union and average girth increment (cm) over two years was calculated using the girth data of 1st year to 3rd year. Single

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leaf area (cm²), plant height (m) from the bud union to the tip of the plant and crotch height (m) from the bud union to the first branching level were recorded. All the plants were test tapped at the age of 2 years and the conformation test of the potential accessions selected was done again in the 3rd year. The tapping system followed was ½S d/3. The data were subjected to analysis of variance for augmented RBD (Federer 1956). The correlations between yield and various growth parameters were computed following the methods of Panse and Sukhatme (1978). Performance of all these genotypes were assessed by ranksum method. The highest rank sum value was treated as rank one and like wise.

RESULTS AND DISCUSSION

The range and general mean values of seven characters in comparison with the four control clones in presented in Table 2. All the genotypes exhibited significantly high variation for the quantitative traits studied except single leaf area. The yield per tree per tap ranged from 0.30 g (RO 2153) to 4.82 g (AC 4394) with a general mean of 0.64 g. AC 4394 was the highest yielder followed by RO 3446 (4.37 g), AC 4111 (3.71 g), RO 2846 (3.59 g), RO 2912 (3.58), AC 159 (3.56 g), RO 2798 (3.48 g), AC 176 (3.20 g) and MT 200 (2.91 g), where as the control clones RRII 105, RRII 208, RRIM 600, PB 260 were recorded 2.20 g., 1.62 g., 2.05 g., 2.48 g. respectively. Annamma *et al.*, (1989), Mercy *et al.*, (1995), Rao *et al.*, (1999) and Abraham *et al.*, (2002) have also reported wide variability in the wild germplasm with respect to certain growth and yield traits in traditional rubber growing region in India.

Girth of the plants after 3rd year of planting ranged from 8 cm (AC 3559) to 20.40 cm (AC 4238) and girth increment from 1.10 cm (MT 2229, RO 5430) to 7.37 cm (RO 2846). Highest girth was recorded in the clone-AC 4238 followed by RO 2846 (20.20 cm), RO 2483 (20 cm), AC 1822 (19.80 cm), MT 200 (19.4 cm), AC 2600 (19.4 cm) and AC 4394 (19.20 cm). Girth increment was the highest in RO 2846 (7.37 cm), followed by AC 4238 (7.28 cm), MT 200 (6.81 cm), RO 1556 (6.62 cm) and AC 4394 (6.55 cm). Genotypes with early growth vigour are highly useful for reducing immaturity period. Wide range of variability was observed for the trait single leaf area which ranged from 33.75 cm² (RO 2790) to 232.50 cm² (AC 3755). AC 3755 (230.24), RO 2355 (216.41), RO 3802 (196.47), RO 3177 (194.66), AC 4166 (191.05) recorded large leaf area. General mean values of the wild accessions in lower than that of all the four control clones.

The plant height and crotch height showed a range of 2.35 in (AC 3559) to 7.50 m (7/118) and 1.75 m (AC 3559) to 5.48 m (AC 4114) respectively. 7/118 (7.50), AC 4238 (7.42), RO 2867 (7.36), MT 200 (7.26), MT 185 (7.23), 7/110 (7.13), RO 3429 (7.08), RO 4476 and RO 3159 (7.06) were the tallest plants. Similary highest mean crotch height was noted in AC 4114 (5.48), AC 3423 (5.32), RO 2483 (4.8), AC 1840 (4.64), AC 3874 (4.57), RO 3803 (4.52), RO 8, RO 3766 (4.40) and RO 3429 (4.35). Similarly, moderate estimates of coefficients of variation for certain growth and yield characters in wild *Hevea* germplasm was reported by Rao *et al.*, (1999 & 2006) and Abraham *et al.*, (2002).

Even though the general mean values of the hybrid controls were higher than the wild accessions except single leaf area, certain individual wild accessions showed high mean values for yield and growth characters particularly in AC 4394 and RO 3446 for yield, AC 4328 and RO 2846 for girth and grith increment, 7/118 and AC 4238 for plant height, AC 4114, AC 3423 for crotch height. Accessions superior to the best control clone for yield and growth characters are shown in Table 3. These genotypes with high yield and higher plant and crotch height coupled with girth and girth increment gives an early indication of fast growth of these wild germplasm which is useful in reducing the immaturity period. These genotypes with high crotch height coupled with good girth indicate their high timber potential. The tendency of wild *Hevea* germplasm to branch at a higher level than the Wickham clones was also reported by Azwar *et al.*, (1995) and Rao *et al.*, (1999). Chapuset *et al.*, (1995) also reported variation in branching behaviour among the wild germplasm. The wide variation observed in the wild germplasm were in accordance with the general expectations that wild and primitive forms from the centre of origin exhibit more variability (Varghese, 1992).

Performance index : In order to identify genotypes with maximum number of desirable attributes, the performance of each of the five characters was pooled using rank sum method. The ranking of each genotype based on parametric relationship of yield and growth traits such as girth, girth increment, plant height, crotch height are shown in Table 4. The rank sum values ranged from 38 to 2660 with a general mean of 1339. Overall performance of each genotype was assessed by summing up the rank values obtained for each of character studied. High rank was recorded in the genotype MT 200 (2660) followed by AC 1822 (2650), AC 4238 (2535), AC 4394 (2533), RO 3778 (2521), RO 3483 (2497), AC 2206 (2456) and MT 187 (2446). These genotypes showed relatively high yield and vigorous growth. The wild accessions RO 4314 (38) and RO 4420 (91) recorded the lowest rank sum value. Balasinha *et al.*, (1988), Mercy (2001) and Rao *et al.*, (2006) also reported similar ranking in Coco and wild *Hevea* accessions respectively while evaluating the genotypes. Top 25 per cent of the potential genotypes identified as best performers are AC 1822, AC 4238, AC 4394, RO 3778, RO 3483, AC 2206, MT 187, RO 90, RO 2846, AC 176, AC 159, 7/110, AC 4149 and MT 4351.

Simple correlation coefficients between pairs of different characters is presented in Table 5. The yield was significantly correlated with girth (0.411), girth increment (0,408) and plant height (0.242). Girth was significantly correlated with the girth increment (0.883), plant height (0.775), crotch height (0.454). Plant height with crotch height (0.605). No significant correlations, however, could be detected between yield and

Table 1 : Provenance wise distribution of accessions.

Provenance	No. of accessions
Acre	223
Mato Grosso	86
Rondonia	229
Others (7 series)	7
Total	545

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Table 2 : Variability for yield and growth related characters in wild *Hevea* germplasm.

Characters	Wild accessions			Control clones				CV (%)
	Minimum	Maximum	General mean	RRII 105	RRII 208	RRIM 600	PB 235	
Yield (g/t/t)	0.30 (RO 2153)	4.82 (AC 4394)	0.64	2.02	1.62	2.05	2.48	108.90
Girth (cm)	8.00 (AC 3559)	19.40 (MT 200)	13.16	14.50	13.58	14.22	17.11	18.85
Girth increment (cm)	1.10 (MT 2229, RO 5430)	7.37 (RO 2846)	3.97	4.82	4.30	4.28	5.73	26.12
Single leaf area (cm^2)	33.75 (RO 2790)	232.50 (AC 3755)	100.97	83.34	91.67	72.61	96.30	28.19
Plant height (m)	2.35 (AC 3559)	7.50 (7/118)	4.94	5.15	4.57	5.48	6.04	23.10
Crotch height (m)	1.75 (AC 3559)	5.48 (AC 4114)	2.6300	2.41	2.22	2.72	3.18	24.39

Note : Figures in parenthesis denotes the name of accession.

Table 3 : Accessions superior to the best control clone for yield and growth related characters.

Characters	No.	Accessions	RRII 105	RRII 208	RRIM 600	PB 235	CD (5%)
Yield (g/t/t)	12	AC 4394 (4.82), RO 3446 (3.92), AC 4111 (3.71), RO 2876 (3.59), AC 159 (3.56), RO 2798 (3.48), AC 176 (3.20), MT 200 (2.91), AC 3986 (2.87), RO 3246 (2.84), RO 2912 (2.66), MT 4351 (2.63)	2.02	1.62	2.05	2.48	1.69
Girth (cm)	20	AC 4238 (20.4), RO 2846 (20.2), RO 2483 (20), AC 1822 (19.8), MT 200, AC 2600 (19.4), AC 4394 (19.20), RO 3976. AC 4339, AC 4102 (18.25), AC 4432 (18.20), AC 176, AC 3557 (18), MT 4351 (17.67), RO 1253 (17.50), AC 2206 (17.33), RO 3653 (17.25), AC 159, RO 3778, MT 4251, MT 2227 (17.20).	14.50	13.58	14.22	17.11	2.89
Girth increment (cm)	21	7.37 (RO 2846), 7.28 (AC 4238), 6.81 (MT 200), 6.62 (RO 1556), 6.55 (AC 4394), 6.55 (RO 1253), 6.51(RO 2483), 6.30 (RO 2914), 6.26 (RO 95), 6.14 (AC 4339 & AC 4102), 6.10 (AC 1822), 6.06 (AC 2600), 6.05 (AC 158), 5.93 (4432), 5.89 (RO 3976), 5.86 (AC 176 & AC 3557), 5.81 (MT 185), 5.80 (MT 2227).	4.82	4.30	4.28	5.73	1.29
Single leaf area (cm^2)	114	(Top fifteen only) AC 3755 (230.24), RO 2355 (216.41), RO 3802 (196.47), RO 3177 (194.66), AC 4166 (191.97), AC 2930 (191.05), AC 4260 (189.79), AC 2203 (186.75), RO 3904 (185.97), RO 3803 (185.19), RO 247 (172.51), MT 932 (171.49), 7-102 (171.45), AC 3857 (170.18), RO 4424 (170.18).	83.34	91.67	72.61	96.30	31.10
Plant height (m)	103	(Top sixteen only) 7/118 (7.50), AC 4238 (7.42), RO 2867 (7. 36), MT 200 (7.26), MT 185 (7.23), 7/110 (7.13), RO 3429, (7.08), RO 4476 & RO 3159 (7.06), MT 187, RO 138, RO 1272, RO 2867 (7), AC 4114, RO 3042 (6.9), AC 1822 (6.87).	5.15	4.57	5.48	6.04	1.29
Crotch height (m)	100	(Top twenty only) AC 4114 (5.48), AC 3423 (5.32), RO 2483 (4.8), AC 1840 (4.64), AC 3874 (4.57), RO 3803 (4.52), RO 8 & RO 3766 (4.40), RO 3429 (4.35), RO 3977 (4.27), AC 1986 (4.17), RO 2793 (4.15), RO 1552 (4.10), RO 2857 (4), AC 2709 & AC 3941 (3.95), RO 138 & AC 157 (3.93), AC 4300 (3.92).	2.41	2.22	2.72	3.18	0.91

PS. Figures in parenthesis indicate mean values.

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Table 4 : Top ranking of wild accessions based on yield and growth parameters.

Accession	Ranksum	Rank
MT 200	2660	1
AC 1822	2650	2
AC 4238	2535	3
AC 4394	2533	4
RO 3778	2521	5
RO 2483	2497	6
AC 2206	2456	7
MT 187	2446	8
RO 90	2442	9
RO 2846	2435	10
AC 176	2431	11
AC 159	2423	12
7/110	2414	13
AC 4149	2361	14
MT 4351	2361	14
Bottom RO 4314	38	549
RO 4420	91	548
MT 3705	135	547
RO 2383	169	546
RO 885	212	545

General mean = 1561.39

leaf area. However, high correlation between yield and girth has been reported in wild *Hevea* germplasm by Madhavan et *al.*, (1996) and Rao and Reghu (2000). Zeng *et al*, (2005) also reported that the existence of significantly high correlations between traits yield and girth and crotch height in wild germplasm.

Morphological, anatomical, biochemical and physiological characters of the rubber tree are ultimately manifested in the volume of latex obtained by tapping and the quantum of rubber it contains. A vigorous habit in the early growth phase of the plant reduces the immaturity period. In general, yield and vigour in *Hevea brasiliensis* are hardly separable (Simmonds, 1989). The present study resulted in the identification of vigorous accessions with wide variability for growth and yield trains. Certain genotypes showed superiority for growth and yield characters which indicates its early vigour and yield.

CONCLUSION

Wide variability was observed for various growth and yield contributing traits. AC 1822, AC 4238, AC 4394, RO 3778, RO 3483, AC 2206, MT 187, RO 90, RO 2846, AC 176 were identified as vigorous genotypes with high yield and girth which will be useful for reducing the immaturity period. The high yielders with good growth identified from this study have potential value for timber. These selections could be further evaluated and incorporated in breeding proprammes for evolving new rubber clones.

Table 5 : Correlation coefficients among six quantitative characters.

Characters	Characters				
	Girth	Girth increment	Single leaf area	Plant height	Crotch height
Yield	0.411**	0.408**	-0.088	0.242**	0.072
Girth		0.883**	0.274**	0.775**	0.454**
Girth increment			0.122*	0.624**	0.347**
Single leaf area				0.246**	0.194**
Plant height					0.605**

**Significant at P < 0.01

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Biosurfactant : A Brief Introduction (Review)

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There is worldwide concern about the pollution caused by libration of stable hydrocarbon in the environment, both from industrial activities and from accidental spill of oil, faciliate the source of considerable polycyclic aromatic hydrocarbon (PAH) contamination. In recent years, attention has to be made for degradation of these contaminations . In this context, Biosurfactants plays a major role. Biosurfactant are the surface active molecules synthesized by microorganism and produced extracellular or on cell surface. They are amphipatic in nature containing both hydrophobic and hydrophilic moiety. The use and commercial application of biosurfactant has increased during the past decade, such as, in food industries, bioremediation of PAH, enhanced oil recovery, cosmetics, therapeutics (immunosuppressive, antibacterial , antifungal and antiviral). Biosurfactant are glycolipids, Lipopetide and lipoprotein, fatty acid, neutral lipid and phospholipids, polymeric and particulate in nature. They are biodegradable, low toxic, biocompatible, stable at extreme pH and temperature. The demands of these biomolecule are increasing day by day due to large application and having unique properties. Hence industrial processes are being developed for high production using cheap raw materials such as oil waste, lactic whey and distillery waste. In spite of high demand, production is not competitive with those of chemical surfactant due to their high production costs and low yield, therefore, attempt has been made to increase yield by using hyper producing mutants, optimizing media constituent and environmental condition. Factors which affect biosurfactants production are carbon sources, nitrogen sources, optimal pH, temperature, agitation and salt concentration.

Keywords : Polycyclic Aromatic Hydrocarbon (PAH), Biosurfactant.

INTRODUCTION

Pollution of Aquatic Environment and soil is the major environment associated problem that harms flora and fauna and cause accumulation of toxic chemicals which amplified in food chain. Although substantial progress have been made to reduce this situation *e.g.*, by using bioremediation technology, but problem still occur due to industrial expansion and incomplete combustion of organic waste. Hence, attention has to be made for degradation of ubiquitous polycyclic aromatic hydrocarbon (PAH). Pollution of soil by tar oil from coal gasification and discharge of petroleum oil from natural oil field, accidental spill of oil, facilitate the source of considerable PAH contamination. Stable aromatic ring, lipophilic nature, insolubility, hydrophobicity are the major factors responsible for persistent of PAH in environment. The PAH concentration at contaminated soil or sediment site has been reported to range from 1 µg/Kg to several percentages by mass (Wilson *et al.*, 1993). The U.S. Environmental protection agency (U.S.EPA) classified 16 PAHs as priority pollutant (Renner *et al.*, 1999). Hence maintenance of sustainable ecosystem urgently requires the development of efficient techniques to reduce pollution.

Attempts have been made to enhance the solubility of PAH by using surfactant. *e.g.*, Triton X-100, Tergiol NPX, Brij 35 and Igepal CA-720 (Edwards *et al.*, 1991). Surfactants are amphiphilic molecule consisting of hydrophilic and hydrophobic parts (Banat *et al.*, 2000). Almost all surfactant currently in use are chemically derived from petroleum which are highly toxic and environmental hazardous. In recent years, interest has been increasing in microbial surfactant (Biosurfactant) due to lower toxicity, higher biodegradability, environmental compatibility, effectiveness at extreme temperature or pH value (Cameotra and Makkar, 1998), produced by microbial fermentation process using cheaper agro- based substrate and industrial waste materials (Patel and Desai, 1997). Biosurfactants are amphipathic in nature, therefore they are accumulate preferentially between fluid phase and reduce surface and interfacial tension (Desai and Banat, 1997). These are structurally diverse group of surface active molecule synthesized by microorganism by different metabolic activities

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and having variety of chemical structure such as Glycolipids, lipopetides and lipoproteins, Fatty acid, Phospholipids, Polymeric and particulate biosurfactant (Sen *et al.*, 2006). The unique properties of these molecules allows their use as worldwide and have several advantages over chemically synthesized surfactant which are mainly derived from petroleum. The major advantages of biosurfactants are - Biodegradability, low toxicity, biocompatibility and digestibility (as used in pharmaceuticals, cosmetics, food industries), specificity, stable activity at extreme of temperature and pH (Kosaric *et al.*, 1992). These surface-active molecules possess a large variety of properties which are widely applicable in industrial process involving wetting, foaming, emulsification, detergency, dispersing or solubilization (Gautam *et al.*, 2006).

Since all surfactants are chemically synthesized which are mainly derived from petroleum, which are toxic, recalcitrant to biodegradation, expansive and insoluble, therefore attention has been made towards commercial production of biosurfactant due to large application such as-microbial enhanced oil recovery, enhanced PAH degradation and bioremediation (Makkar and Rockne, 2003), Crude oil drilling lubricant, food industries, therapeutic and biomedical (Eun *et al.*, 2008), cosmetic and soap formulation (Lourith and Kanlayavattanakul, 2009).

Application of biosurfactant in PAH bioremediation : In recent year, attention towards potential use of biosurfactant for enhanced PAH degradation rates by increasing microbial accessibility to insoluble PAH. Based on the effect of surfactant on the availability of organic compound, the following mechanism can be explained (Makker and Rockne, 2003).

1. **By increased surface availability -** Surfactant increass in contact area by dispersion of non-aqueous phase liquid organics which cause by reduction in the interfacial tension between the aqueous phase and non-aqueous phase.
2. **Increased solubility of pollutants -** Surfactant cause micelles formation that accumulate high concentration of hydrophobic organic contaminants and cause solubilization.
3. **Facilitate transport of the pollutant from solid phase-** Increase in facilitate transport can be caused by lowering of surface tension, increase interaction of pollution with surfactant.

Schippers *et al.*, (2000) suggested three approaches for the biodegradation of PAH by using biosurfactants :

1. As first approach microbes are able to directly uptake PAH from micelle.
2. Secondly, Biosurfactant help to increase the mass transfer rate of pollutants to aqueous phase so that microbes can be use further.
3. Thirdly, they facilitate the direct contact between cells and non-aqueous phase liquid by changing the hydrophobicity property.

A number of techniques are being used for rapid screening of hydrocarbon degrading microorganism such as, Thin layer chromatography (Matsuyama *et al.*, 1991), Drop collapse method (Jain *et al.*, 1991), Hemolytic measurement (Mulligan et al., 1984), Estimation of emulsification index value (E-24) and Surface tension measurement by using tensiometer.

Factors affecting biosurfactant production :

1. Carbon source - Different water soluble carbon sources such as glucose, mannitol, glycerol, ethanol, vegetative oil are used. A variety of cheap raw materiais have been reported as carbon source for biosurfactant production (Sen *et al.*, 2006) *e.g.*, starchy substrate, oil waste, lactic whey, distillery waste shows some of the inexpensive raw material for biosurfactant production.

2. Nitrogen source - The nitrogen source in medium also affect biosurfactant production as reported by some author. Copper and Zajic, 1980 observed ammonium salt and urea were preferential nitrogen source for biosurfactant production by *Arthrobacter parafineus*, whereas nitrate support maximum surfactant production in *P. aeruginosa* and *Rhodococcus* sp. Nirogen limitation cause increased biosurfactant production in *Ustilago maydis* (Hewald *et al.*, 2005), *P. aeruginosa, C. tropicalis and Nocardia* strain. Guerra Santos *et al* shows maximum rhamnolipid production after nitrogen limitation at C : N ratio of 16 : 1 to 18 : 1 and no surfactant production below C : N ratio of 11 : 1, where the culture was not nitrogen limited.

3. Environmental factors -

A. pH - Guerra-Santos *et al.*, 1984 observed maximum rhamnolipid production in *Pseudomonas* sp. at pH range 6 to 6.5 while penta and disaccharide lipid production in *N. corynbacteroides* is unaffected in the pH range of 6.5 to 8.
B. Temperature - Benat *et al.*, 1995 showed *Bacillus* sp. grew and produced biosurfactant at temperature above 40°C. Most of the biosurfactant are stabe at higher temperature.
C. Agitation speed - Biosurfactant production increase with increased agitation and aeration rates in most of microorganism and yeast.
D. Salt concentration - Salt concentration were not affected at the concentration upto 10% (W/V) while Banat *et al.*, 1995 showed slight reduction in critical micelle constant.

Bioprocess development : An efficient and economical bioprocess is the bottleneck for every profit making biotechnology industries. Although interest in biosurfactant production has increased as they are potential candidate for many commercial application in petroleum (Microbial enhanced oil recovery), cosmetics, foods and pharmaceutical industries. However, applications of these biomolecules are limited by their cost of production, which can be reduced by optimizing the constituent of different media and by using cheap agro-industrial waste. Hence optimal addition of media component and optimal cultural condition are primary key steps for biosurfactant commercialization. The classical method of medium optimization involves changing one variable at a time while keeping the others at fixed levels. This method is laborious and time consuming and does not guarantee determination of optimal condition. Therefore statistical

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optimization based on response surface methodology has been frequently used for optimization. Response surface methodology mainly consist of a group of empirical techniques to the evaluation of relations existing between a group of controlled experimental factors such as media composition, temperature, pH, oxygen uptake and the measured responses, according to one or more selected criteria which based on mathematical and statistical techniques such as fractional factorial designs, Central composite design, Data analysis (Rodrigues *et al.*, 2006). The significance of the result is usually checked by F-test and analysis of variance (ANOVA) used to validate the regression conefficient.

Downstream processing for product recovery : An efficient and economical purification process is backbone of any commercial profit-making biotechnology industry, About 60% of total production costs are invested for efficient downstream process (Sen *et al.*, 2006). Due to presence of hydrophobic moiety, organic solvent extraction process in generally used for biosurfactant recovery. Organic solvent such as Chloroform-methanol, dichloromethane-methodnol, butanol, ethyl acetate, acetic acid are mainly used at acidic pH of 2.0. Among these methanol, acetone and chloroform are toxic in nature, therefore tertiary-butyl ether are generally used now a days to recover biosurfactant produced by *Rhodococcus*. Biosurfactant posses a property of insolubility at low pH and at high centrifugal force, so recovery process using acid precipitation and centrifugation are most common methods, whereas for high purity requirement (*e.g.*, theaurapatic purpose) ion-exchange chromatography, membrane ultrafilteration and adsorption on activated carbon or polystyrene resins are widely used. For the recovery of polymeric or protein rich biosurfactant, ammonium sulfate precipitation are generally recommended.

CONCLUSION

The unique properties of biosurfactant have led to a potential candidate for wide range of application in bioremediation, enhanced oil recovery, immunotherapy, cosmetics etc. At present, the productions of microbial surfactant are not very efficient for commercial purpose due to low yields and high production costs. Although significant progress has been made for high yield by using optimization of media, environmental condition and by using cheap substrate e.g., agro-waste, but progress are still very less and price are not competitive with those of chemical surfactant. This can be possible and successful with the better knowledge of genetic constituent of surfactant producing microbes so that selections of hyper producing strain are easier to achieve. In spite of this hidden mystery, we do not have adequate information about genetic regulation of surfactant producing microbes. Therefore, efforts should be made to develop hyper producing starin by using mutation, genetic engineering and develop an efficient bioprocess technique for high yield.

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Development of Protocol for Raising Mulberry Saplings from Soft Wood and Semi-soft Wood Cuttings

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Rapid multiplication and spread of superior crop plant varieties within the shortest possible time is as essential as developing them, to justify the need for invesiment of resources in breeding programmes. In sericulture, it is often felt that the superior varieties of food plants and silkworms reach the end users taking a long time leading to under utilization of the existing potentials.

The present study explored the possibilities of raising mulberry saplings by using softwood and semi-softwood shoots to enhance the rate of multiplication. Forty five to sixty days old shoots of V-1 mulberry variey were used as seed material. Cuttings were prepared from the top 1/3rd, middle 1/3rd and bottom portions of the shoots. Two sets of cuttings, one set with leaves intact and the other without leaves were prepared. These cuttings were treated with 20 ppm NAA and planted in nursery beds prepared under tree shade. The nursery beds were covered with polythene sheet followed by gunny cloth. Percentage rooting and number of roots per cuttings were recorded after 45 days of planting. Considerable variability in rooting was recorded between cuttings planted under tree shade and those in nursery beds with polythene covers. The highest rooting was observed in softwood cuttings, with leaves intact, which were planted under controlled conditions. The study revealed that multiplication through soft and semi-soft wood cuttings could increase the multiplication rate.

Keywords : Mulberry, multiplication, Softwood, Hardwood, Cuttings.

INTRODUCTION

Mulberry (*Morus* spp.) is a perennial crop, once established, consistently yields quality leaf for a period of about 15-20 years. Mulberry is predominantly propagated through stem cuttings, a practice followed since time immemorial. The stem cuttings, depending upon the nature of wood have been termed as hardwood, semi-hardwood and softwood. Besides the edaphic and climatic conditions, factors such as type of wood, stage of stock plant growth and time of plantation play important role in rooting and subsequent survival of cuttings (Hartmann and Kestar, 1986). In general, hardwood cuttings collected from six to eight months old (after the last pruning) mulberry shoots are recommended for planting (Krishnaswami, 1986, Dandin *et al.*, 2002). The contribution of superior varieties is the most satisfying and most inexpensive of all the efforts to improve productivity and quality of mulberry. In the recent years, due to the development of superior mulberry varieties and cultivation technology, the leaf productivity of 60-70 metric tonnes/hectare/ year has been achieved in the country. However, rate of spread of such superior varieties has remained slow.

The major reason for slow rate of spread of new mulberry varieties is inadequate availability of seed material in time and use of only hard wood (>6 months old) cuttings for planting. High degree of adoption of shoot rearing technology involving cutting the whole shoots for feeding silkworms, a practice that is beneficial in terms of higher returns through low cost of production and better hygiene, has lead to non-availability of seed cuttings (hard wood) from commercial gardens. This has necessitated establishment of exclusive seed gardens for supply of seed cuttings for raising new plantations.

In the traditional hardwood multiplication system, seed cuttings are obtained from a seed garden at the most twice in a year, leading to time lapes between evolution of an improved mulberry variety and its exploitation in the farmers' fields. Multiplication of plant types through softwood cutting is practiced in a variety of crops (Ercisli & Read, 2001; Radwan *et al.*, 1989; Pijut and Moore, 2002) including temperate mulberry varieties as in Peoples Republic of China and Japan (Mogili, 2000). However, the procedure has not been standardized for tropical mulberry varieties. Therefore, studies were carried out to standardize the propagation method by using soft and semi-soft (green) wood cuttings. Multiplication through soft and semi-softwood cuttings offers an opportunity to obtain planting material six times in a year from a single

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seed garden thereby improving the rate of multiplication. High yielding mulberry variety V-1, developed at Central Sericultural Research and Training Institute, Mysore, which is the most sought after variety among the farmers in the irrigated tracts of South India was selected for standardizing the multiplication technology.

MATERIAL AND METHODS

Exclusive seed garden is a prerequisite for softwood multiplication. The study was conducted at the Central Sericultural Research and Training Institute, Mysore, which has red loamy soil. The soft and semi-softwood cuttings were obtained from a seed garden of V-I variety, established with (90 + 150) x 60 cm spacing and maintained under assured irrigated conditions with recommended inputs of 20 tonnes of FYM and NPK @ 300:120:120 Kg/ha/ year (Dandin *et al.*, 2000).

Preparation of cuttings : Soft wood shoots were harvested from the seed garden between 45th and 60th day after pruning (DAP) to prepare cuttings. Three types of cuttings *viz.*, cutting from top 1/3rd portion, cuttings from the middle 1/3rd portion and the remaining bottom portions of the shoots were prepared separately. 15-20 cm long cuttings with one or two leaves intact at the top were prepared using secateur. The upper ends of the cuttings were cut horizontally above a bud and the lower ends obliquely below a bud (Fig.1) to avoid drying at the top and increase surface area for rooting at the lower end. The cutting were bundled with 20 to 30 cuttings in each bundle with the lower cut ends arranged in the same plane and subjected to rooting expediting treatment. The lower portions (2 to 3 cm) of the bundled cuttings were dipped in 20 ppm NAA for 24 hours to enhance rooting (Mogili, 2000). The treated cuttings were planted in nursery beds after washing in water.

Preparation of nursery beds: Land with fertile soil and assured irrigation was selected for the purpose. The beds were prepared after ploughing to mix adequate quantities of sand, compost or FYM. Raised beds of 5 cm height, 70 cm width and 400 cm length were prepared.

Covering the beds: Covers made of transparent polythene sheets supported on bamboo frames in the form of low tunnels were employed to cover the nursery beds and provide high moisture (Fig. 2). The height of the tunnel at the center was 50-60 cm above the bed. Jute fabric (Gunny cloth) was spread over the polythene covers to provide required shade to the nursery beds (Fig. 3). Care was taken to provide optimum temperatures of 25 to 30°C and a light intensity of about 2000 lux. An additional layer of Gunny cloth was used over the polythene covers wherever the nursery beds were not in the tree shade. Simultaneously, another batch of cuttings from the top middle and bottom portions of the stem were planted under tree shade without the poly-tunnel covering and with and without leaves on the cuttings.

Plantation: Softwood cuttings treated with root promoting hormone were inserted into the beds during early hours and in the evenings to avoid bright sunshine. Cuttings were planted in 5 rows in each bed with an inter-raw space of 15 cm and inter cutting space of 10 cm in each row. About 5 cm of the base of cuttings was inserted into the ground.The beds were irrigated with sprinkler and the polythene covers placed over the beds.

Post planting care : The beds were moistened only when they appeared dry, by irrigating the channels around the beds, thereby avoiding excessive moisture levels. As far as possible the observations were made without opening the covers, through the polythene, after removing the gunny cloth.

Removal of shade: One or two cuttings on the edges of the beds were dug out to observe for rooting after 35 days of planting. The cuttings were maintained for 45 days, when more than 10 roots appeared per cutting (Fig. 4). The polythene sheets were removed partly on one side on the 46th day and entirely on the following day (Fig. 5).

RESULTS AND DISCUSSION

Data pertaining to survival and number of roots per sapling were recorded (Table 1). Results clearly indicated that the nursery raised under controlled conditions (poly-tunnel condition) resulted in higher survival and developed more number of roots.

Survival and rooting in all the three types of cuttings was relatively higher under controlled environment of poly-tunnel (78.20 - 88.70%). The cuttings with leaves intact showed higher percentage of survival and average number of roots per cutting compared to the same type of cuttings without leaves. The observations are in conformation with the observations of Honda (1970), who reported positive correlation between the number of leaves on the cuttings and the weight of new roots in mulberry softwood cuttings. Whereas, in beds without polythene cover, the difference in survival between leaved and defoliated cuttings was not significant, where the leaves left intact on the cuttings withered away.

The survival percentage was highest in softwood cuttings with leaves intact followed by semi-softwood cuttings from the middle 1/3rd portion of the stem and those from the bottom 1/3rd portion. On the contrary, in beds without the poly-tunnel covering, the survival percentage was highest in cuttings prepared from the bottom portion of the stem, followed by those the middle and top portions.

Nanda and Kochar (1985) attributed the rooting potential of young cuttings to high nutritional level and high content of rooting co-factors and growth promoters in them. Fordham (1972), opined that by modifying and adapting certain components of the nursery raising system, horticulturists can propagate easily through softwood cuttings that previously were considered difficult. Construction of polyethylene propagation chamber may be highly useful in raising the softwood nursery. It is therefore suggested that softwood cuttings can be utilized for propagation of mulberry varieties in general and V-1 variety in particular.

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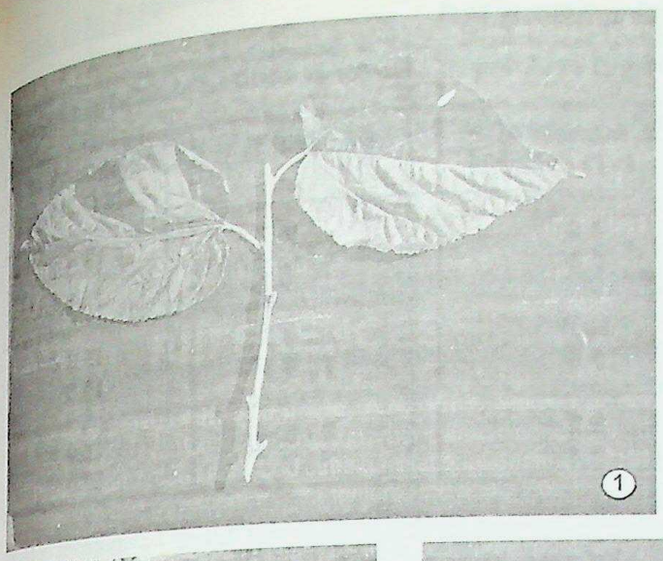

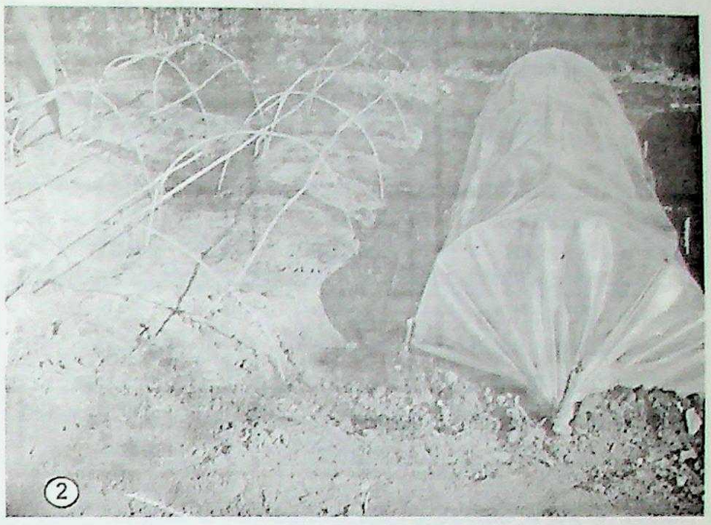

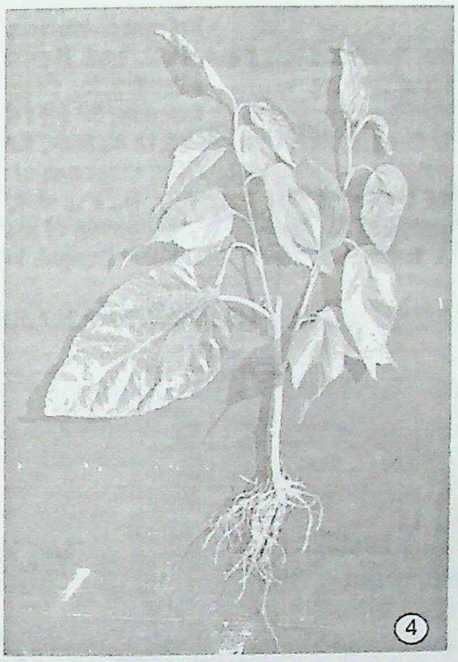

Legends to Figures

Fig. 1. Soft wood cuttings with the upper ends cut horizontally above a bud and the lower ends obliquely below a bud.

Fig. 2. Tunnels made of transparent polythene sheets supported on bamboo frames to cover the nursery beds and provide required environment.

Fig. 3. Jute fabric (Gunny Cloth) spread over the polythene covers to provide required shade to nursery beds.

Fig. 4. Soft wood cuttings with profuse rooting after 45 days of planting.

Fig. 5. Saplings grown from soft wood cuttings after 45 days.

The data clearly indicated that it is possible to achieve high rate of multiplication through soft and semi-softwood cuttings by adopting the poly-tunnel method to take up at least 6 multiplication cycles in a year.

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Table 1 : Survival of softwood cuttings after 40 days of planting (Variety V-1).

Type of seed cutting	Nature of cuttings	Under tree shade without covering		Under controlled shaded conditions	
		Survival %	Av. number of roots / sapling	Survival %	Av. number of roots / sapling
Top 1/3rd portion of Shoot - Softwood	Leafy Leafless	12.3 8.3	8.6 8.3	88.7 78.2	16.3 11.8
Middle 1/3rd portion of Shoot - Semi - softwood	Leafy Leafless	33.8 34.3	10.4 8.9	82.6 78.9	15.8 10.3
Bottom 1/3rd portion of Shoot	Leafy Leafless	66.7 66.8	9.2 9.4	83.8 78.7	12.6 12.6

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In Vitro Embryogenesis and Multiple Shoot Formation in *Eclipta alba* (L.) Hassk

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The recent years has witnessed a remarkable awareness and progress towards esthetic utilization of the medicinal plants ensuring conservation and harnessing processes to be mutually reciprocating. *Eclipta alba* (L) Hassk. (ver. Bhringraja) is one of the most important medicinal plant of the Indian Meteria Medica (Ayurveda, Unani, Siddha Horopathy etc.) The present communication outlines a rapid method of callus induction, embryogenesis and organogenesis leading to multiple shoot formation using shoot apical meristem explant. The full and the half salt strength MS were supplemented with varying concentration and combination of auxins (NAA) and cytokinins (BAP) were investigated for callus induction, embryogenesis and multiple shoot formation with or without coconut milk (CCM) supplementation. The effects of combination *vis-a-vis* concentration of auxins and cytokinins and supplementation of CCM are outlined and discussed that affect the process of embryogenesis and multiple shoot formation. The optimum callus was recorded in 3.0 mgl^{-1} NAA while the maximum number of embryoids was recorded NAA (0.5 mgl^{-1}) + BAP (2.5 mgl^{-1}). The maximum number of multiple shoot was recorded in NAA + BAP (0.5 + 3.0 mgl^{-1}) but when full strength MS was supplemented with CCM there was remarkable increase both in the number of shoot $explant^{-1}$ as well as significant increase in the growth rate.

Keywords : Auxins, NAA, Cytokinins, BAP, Coconut Milk (CCM), Callus, Embryogenesis, Multiple shoot.

Abbreviations : MS, Murashige and Skoog; NAA, α-Naphthalene Acetic acid; BAP, 6-Benzyl Aminopurine; CCM, Coconut Milk; E-oids, Embryoids.

INTRODUCTION

The relevance of the complementary and alternative medicine system (CAMS) in the recent years have gained renewed interest and revival due to the fact that they are safe, economical and above all these provide panacea to people suffering from ailments that have limited options of treatment. *Eclipta alba* (Lin.) Hassk. syn. *Eclipta prostrata* (Lin.), *Eclipta erecta* (Lin.), (Bhringaraj, Keshraj) Asteraceae, is known popularly as 'Bhengri' by the tribal of Jharkhand. The plant occupies an important place among the medicinal plant in the Indian Metria Medica like the Ayurveda, Unani, Siddha and Horopathy (tribal medicine). The curative and cosmetic application of the crude, aqueous decoction and alcoholic extracts are well documented that dates back to antiquity. The phytochemistry of the plant is diverse and contains triterpene saponin (eclalbatin) (Upadhaya, Pandey, Jha, Pandey, 2001), dithienylacetylene esters (Singh and Bhargava, 1992), and analgesic alkaloid (Sawant, Isaac, Narayannan, 2004), taraxastane glycosides (Yahara *et al.* 1997) and host of other biologically active substances which are either disease preventive or curative particularly relating to neurological and psychiatric (Zhang, 2004; Thakur and Mengi, 2005), dermatologcial wound healing (Sharma and Sikarwar, 2008), osteoporosis (Lee *et al.*, 2008; Singh and Bhargava, 1992), hepatotoxic and renal dysfunction and urological complications (Franca, Bertoni and Pereira, 1995). Literatures pertaining to the *in vitro* studies using full strength MS salts are fairly voluminous describing, rapid and simplified protocols for the micropropagation (Borthakur *et al.*, 2004; Gawde and Paratkar, 2005; Dhaka and Kothari, 2005; Neeti and Kothari, 2005; Ray and Bhattacharya, 2008; Sayeed Hassan *et al.*, 2008). In the present communication the effects of Coconut Milk (CCM) (endosperm) in the induction of callus, embryoids and shoot formation *vis-a-vis* growth regulators in varying combinations and concentrations (NAA, BAP) using full strength MS is discussed and highlighted.

MATERIAL AND METHODS

The plants of *Eclipta alba* (Lin.) Hassk. (Bhringaraj) was identified and collected locally from perennial wet places (municipal drains and other water bodies) and maintained in the earthen pots (large sized) in the departmental herbal garden. Freshly harvested plants were removed of all the lateral appendages (leaves, lateral buds). Young shoots below the growing tips were cut between two successive internodes with sharp blade to remove single nodes used as explants. The explants were washed in running tap water followed by further rinsing in distilled water and surface sterilized in 50% v/v

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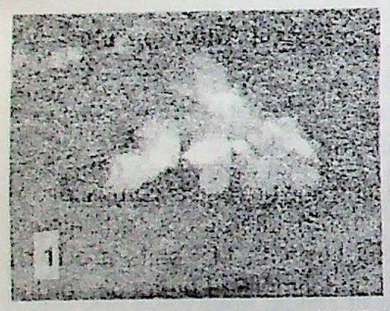
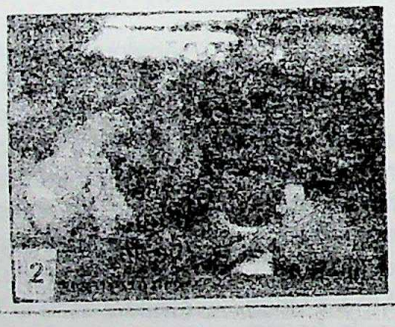
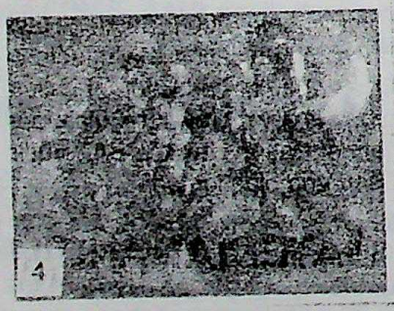
Table 1 : Effects of growth regulators and coconut milk supplementation in MS on callusing, embryogenesis and multiple shoot formation from shoot apical meristem explant in *Eclipta alba* (Lin.) Hassk.

Growth Regulators Auxin + CK ± CM (Auxin & CK in mgl^{-1})			Callus Development with Embryoids			
			Mean no. of explant Callused	Mean No. of E-oids ($explant^{-1}$)	Days Taken	Mean No. of callus with Multiple Shoots
NAA + BAP + CCM*						
0.0	0.0	-	-	-	-	-
0.5	0.0	-	10	-	28 ± 3	-
1.0	0.0	-	23	-	28 ± 2	-
1.5	0.0	-	67	-	24 ± 3	-
2.0	0.0	-	77	-	23 ± 3	-
2.5	0.0	-	83	-	23 ± 2	-
3.0	0.0	-	92	-	20 ± 3	-
0.0	0.5	-	-	-	-	-
0.5	1.0	-	14	-	28 ± 5	7
0.5	1.5	-	20	0.2	28 ± 3	17
0.5	2.0	-	17	0.8	27 ± 3	31
0.5	2.5	-	17	1.3	27 ± 3	53
0.5	3.0	-	16	0.7	27 ± 3	57
0.0	0.5	+	-	-	-	-
0.5	1.0	+	14	0.5	23 ± 3	32
0.5	1.5	+	21	0.9	23 ± 3	51
0.5	2.0	+	19	2.3	24 ± 4	67
0.5	2.5	+	19	3.8	22 ± 4	72
0.5	3.0	+	18	5.7	24 ± 2	78

CCM* = MS supplementation with CCM to the tune of 15 % v/v.







Legends :

Fig. 1 : Three weeks old shoot apical explant showing initiation of callus (MS + 3.0 mgl^{-1} NAA).

Fig. 2 : Four weeks old callus showing fresh callus (white), yellow (slightly old) and brown callus (approaching senility) due to the presence of secondary metabolites (MS + 3.0 mg l^{-1} NAA).

Fig. 3 : More than four weeks old callus with number of embryoids (MS + 0.5 mgl^{-1} NAA + 2.5 mgl^{-1} BAP).

Fig. 4 : Four weeks old callus mass with multiple shoots (MS in 15% CCM + 0.5 mgl^{-1} NAA + 3.0 mgl^{-1} BAP).

Fig. 5 : Complete shoot development from callus mass (MS in 15 % CCM + 0.5 mgl^{-1} NAA + 2.5 mgl^{-1} BAP).

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ethanol for 30 seconds and agitated vigorously in 0.1 % w/v mercuric chloride for 10 min. The explants were transferred to full strength MS media fortified with growth regulators auxins (NAA) and cytokinins (BAP) in different combinations and concentration (NAA + CK), with and without CCM. One set of experiments the MS contained NAA in variable concentration (from 0.5 mgl^{-1} to 3.0 mgl^{-1}) without CK and CCM (Table 1). In another set MS was fortified with minimal (0.5 mg l^{-1}) concentration of NAA and the CK concentration varied (0.5 to 3.0 mgl^{-1}) without CCM. In the third set of experiments the minimal concentration (0.5 mgl^{-1}) of NAA but CK concentration was variable and the media contained 15 % v/v CCM. The cultures were transferred to freshly prepared media for sub culturing after every 20 to 25 days. The pH of the medium was adjusted at 5.8 (1N NaOH or/and 1N HCl) and the incubation temperature of 23 ± 2°C under fluorescent lighted condition in culture room. Data were collected from three replicates to ascertain the repeatability and authenticity. Half strength MS media was ignored as it was not found suitable for callusing, embryogenesis and multiple shoot formation.

RESULTS AND DISCUSSION

The recent few decade has witnessed striking implosion of information, due to renewed awareness and revival of faith in the traditional system of medicine and cure (Zhang, 2004) particularly in India, China and African nations. The results set out in Figure 1 to 5 and Table 1 were indicative of the fact that full strength MS supplemented with NAA was extremely effective in inducing (within limits) callus in all concentration and the callusing time gradually decreased with the corresponding increase in the mean number of explants displaying callus. Similar results were not recorded in half strength MS and hence all results pertains to data collected using full strength MS media. However, NAA alone failed to initiate embryo embryoid and shoot formation even after prolongation of incubation period (4 - 5 weeks). Longer culture period results in retardation and browning of the callus. Hence in all treatment sub-culturing was done after every three to four weeks. However, callus embryoids and multiple shoots were concomitantly recorded when both the NAA and BAP used in combination in which the former (NAA) was kept constant at the minimal concentration (0.5 mgl^{-1}) but the concentration of BAP varied between 0.5 to 3.0 mgl^{-1}. In these there was significant increase in the mean number of explant displaying callus, embryoids and multiple shoots. The optimum results were obtained in 0.5 + 2.5 mgl^{-1} respectively of NAA and BAP with lower number of multiple shoots (Dhaka and Kothari, 2005; Neeti and Kothari, 2005; Gawde and Paratkar, 2004; Ray and Bhattacharya, 2008; Borthakur *et al.*, 2004; Sayeed Hassan *et al.*, 2008). These parameters significantly increased when similar combinations and concentration of NAA + BAP used in combination with CCM containing MS media, which was for the first time tried on *Eclipta alba*. The results reflected to the neutraceutical effects of CCM that not only displayed enhancement in the explants displaying callus, embryoids and multiple shoots but also significantly reduced the culture period. the CCM in these experiments displayed neutraceutical effects on the micropropagation of *Eclipta alba* that enhance growth and developmental activities attributable to nursing, nurturing and nutritional properties of CCM over other synthetic medium.

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Preliminary Phytochemical Investigation and Antimicrobial Activity the Leaves of *Clerodendrum phlomidis*

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The hot water, methanol, ethanol, chloroform and petroleum ether extracts of the leaves of *Clerodendrum phlomidis* were screened for phytochemicals and their antimicrobial activity.Phytochemical analysis reveals the presence of alkaloids, saponins, terpenoids, steroids, tannins, phlobatannins, aminoacids, reducingsugar, lignin, inulin, glycosides, monosacharides, proteins and cardiac glycosides. The antibacterial assay by disc diffusion method showed that hot water extracts were most active against all the six test organisms than other solvent extracts. The antifungal activity against two fungal species found to be decreased as the concentration of the extract increased.

Keywords : Phytochemicals, Antibacterial activity, Antifungal activity, *Clerodendrum phlomidis.*

INTRODUCTION

Plants have been an essential part of human society since the start of civilization. During the Vedic period great importance was given to plants. Rig-Veda and Atharvaveda described medical plants. Around 250 drugs have been identified from such descriptions [Srinivasan *et al.*, 2001]. The medicinal values of the plants have some chemical substance called as phytochemicals that produce a physiological action on the human body [Hill, 1952].

The genus *Clerodendrum* belongs to the family Verbenaceae is very widely distributed in tropical and subtropical regions of the world. More than five hundred species of the genus are identified till now, which include small trees, shrubs and herbs. The leaves are opposite, deltoid, ovate, hairy from below and wavy. The genus is being used as medicines specifically in Indian, Chinese, Thai, Korean, Japanese systems of medicines. This plant has been used to treat dysentery, syphilis, bronchitis, cancer, jaundice and hypertension. With this background, the study was designed to screen for the presence of phytochemical and antimicrobial activities in the leaves of this plant.

MATERIAL AND METHODS

Plant Materials : The leaves of *Clerodendrum phlomidis* were collected from Keeranoor, Trichy. The herbarium specimen was deposited in the Rapinaet Herbarium, St. Joseph's College, Trichy, Tamil Nadu, India.

Preparation of Extracts : The leaf of the plant materials was allowed to air dry and afterwards powdered it with the help of hands. The solvent used for the preparation of extracts were ethanol, methanol, hot water, chloroform and petroleum ether.

50 grams of plant material were dissolved in 250 ml of solvent [ethanol, methanol, hotwater, chloroform and petroleum ether] and kept in a mechanical shaker for overnight. The obtained extracts were filtered with Whatmann No-42 filter paper (125 mm) and stored in an airtight bottle and kept at 4°C. These extracts were used for experimental analysis [Kumar *et al.*, 2007].

Phytochemical Investigation : The preliminary phytochemical investigations were studied as per the method of Kokate (1994).

DETERMINATION OF ANTIBACTERIAL ACTIVITY

Antibacterial Assay : The plant extracts were tested for antimicrobial activity using agar disc diffusion assay [Bauer *et al.*, 1996] using strains of bacteria *Viz. Escherichia coli, Bacillus* species, *Pseudomonas* species, *Klebsiella* species, *Staphylococcus* species and *Proteus* species. Petri plates containing 20 ml of Mueller Hinton agar medium were swabbed with 24 hours old culture of the above selected bacterial strains. The sterile disc impregnated with various concentration of extract such as 500µl, 750 µl and 1000 µl. The discs were air dried.

The prepared disc containing various concentration of plant extract was placed individually on the bacterial lawn. Each plate contains three discs containing extract in varying concentration and one disc containing DMSO used as a

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Table 1 : Preliminary phytochemical investigation of Hot water, Methanol, Ethanol, Chloroform and Petroleum ether extracts of *Clerodendrum phlomidis*.

S. No.	Tests	Hot water	Methanol	Ethanol	Chloroform	Petroleum ether
1.	Alkaloids	+	+	-	-	-
2.	Flavonoids	-	-	-	-	-
3.	Saponins	+++	-	-	-	-
4.	Tannins	++	-	-	+	-
5.	Cardiac glycosides	-	-	-	-	+++
6.	Phlobatannins	++	-	-	-	-
7.	Steroids	-	+++	+++	+++	+++
8.	Terpenoids	+++	-	-	-	-
9.	Triterpenoids	-	-	-	-	-
10.	Amino acids	+++	-	-	-	+
11.	Reducing sugars	-	+	+++	++	-
12.	Fats and fixed oils	-	-	-	-	-
13.	Lignin	+++	+	+	++	-
14.	Inulin	+++	-	+	-	-
15.	Glycosides	+	-	-	-	-
16.	Monosaccharide	+	-	-	-	-
17.	Proteins	+	++	+	+++	+

+++ indicates highly positive, ++ indicates sparingly positive, + indicates weakly positive, - indicates negative

Table 2 : Antibacterial Activity for Hot Water Extract of *Clerodendrum phlomidis*.

S.No.	Organisms	Zone of Inhibition (mm)			Positive control	Negative control
		500µl	750µl	1000µl		
1.	*Escherichia coli*	16	25	27	11	-
2.	*Proteus* species	12	13	15	20	-
3.	*Klebsiella* species	17	25	26	16	-
4.	*Bacillus* species	16	23	25	14	-
5.	*Staphylococcus* species	15	16	18	15	-
6.	*Pseudomonas* species	16	20	24	17	-

Table 3 : Antibacterial Activity for Ethanol Extract of *Clerodendrum phlomidis*.

S.No.	Organisms	Zone of Inhibition (mm)			Positive control	Negative control
		500µl	750µl	1000µl		
1.	*Escherichia coli*	9	10	12	11	-
2.	*Proteus* species	12	13	15	20	-
3.	*Klebsiella* species	11	18	19	16	-
4.	*Bacillus* species	11	15	16	14	-
5.	*Staphylococcus* species	11	12	13	15	-
6.	*Pseudomonas* species	-	-	-	17	-

negative control. This method was followed for all the five extracts. The culture plates were incubated for 24 hours at 37°C for the zone of inhibition.

Antifungal Assay : The streak plate assay was generally used to obtain discrete colonies and pure colonies. This assay can be used to determine the growth inhibition of fungi by plant extracts. The concentration of the extract used for antifungal assay was 100µl, 200µl, 300µl, 400µl, 500µl, 600µl, 700µl, 800µl, 900µl, 1000µl. 20 ml of potato dextrose agar medium was prepared and sterilized separately and 100 µl of extracts was added and poured on petriplate. This indicate that the

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Table 4 : Antibacterial Activity for Methanol Extract of *Clerodendrum phlomidis*.

S.No.	Organisms	Zone of Inhibition (mm)			Positive control	Negative control
		500µl	750µl	1000µl		
1.	*Escherichia coli*	13	17	18	11	-
2.	*Proteus* species	14	15	17	20	-
3.	*Klebsiella* species	20	21	22	16	-
4.	*Bacillus* species	11	20	23	14	-
5.	*Staphylococcus* species	12	17	21	15	-
6.	*Pseudomonas* species	16	20	24	17	-

plate contain 100 µl of extract along with agar medium. This was followed upto 1000 µl concentration.

With the help of sterilized loop, the organism such as *Penicillium* species and *Aspergillus* species were streaked on the surface of the agar plates. The effect of the plant extract was expressed as the growth variation found in the agar plates after incubation at room temperature for 72 hours. This method was followed for all the five extracts of *Clerodendrum phlomidis*.

RESULTS AND DISCUSSION

The phytochemicals found t be present on the leaves of *Clerodendrum phlomidis* were alkaloids, saponins, terpenoids, steroids, tannins, phlobatannins, aminoacids, reducing sugar, lignin, inulin, glycosides, monosaccharides, proteins and cardiac glycosides. The results were tabulated in Table 1. Table 2 showed that the aqueous extract had good effect of antibacterial activity against *Escherichia coli, Klebsiella* species, *Bacillus* species, *Pseudomonas* species., and a little lesser effect on *Proteus* species and *Staphylococcus* species. It was seen that the ethanol extract had a lesser antibacterial activity on *Proteus* species, *Klebsiella* species, *Bacillus* species, *Staphylococcus* species, and a little lesser effect on *Escherichia coli*. But the ethanol extract has no effect against *Pseudomonas* species it was shown in Table 3. In Table 4, it was seen that the methanol extract had moderate activity on *Klebsiella* species, *Bacillus* species, *Staphylococcus* species and least activity observed on *Escherichia coli, Proteus* species and *Pseudomonas* species. The chloroform and petroleum ether extract had no antibacterial activity for both gram positive and gram negative organisms.

The antifungal activity of the leaves of *Clerodendrum phlomidis* were found to be decreased as the concentration of the extract increased for each microlitre. The concentration of the different extracts will inhibit the growth of fungal pathogens such as *Aspergillus* species and *Penicillium* species. Singh *et al.*, [2004] pointed out that the extract taken using water as solvent showed greater inhibitory activity than methanol extract.

Thaakur [2006] reported that the chloroform extract of *Adhatoda vasica* leaf did not show any antibacterial activity against *Escherichia coli, Proteus* species, *Bacillus* species, *Staphylococcus* species and *Pseudomonas* species. Ramasamy *et al.*, [2004] reported that petroleum ether has no antibacterial effect. Sathishkumar *et al.*, [2007] showed that the phytochemicals present on *Elaeocarpus ganitrus* Roxb. and *Canthium parviflorum* Linn. Leaves contain alkaloids, saponins, terpenoids, steroids, tannins and glycosides. The results were almost the same as report given above for *Clerodendrum phlomidis*.

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Comparative *In Vitro* Efficiency of *Cassia tora* and *Cassia sophera* in Mitigating Fungal Infections

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Cassia tora and *Cassia sophera* belonging to family Caesalpinaceae are wild and edible plants used in traditional antifungal medicine in tropical and subtropical countries. They are commonly found growing in wastelands. In the present study antifungal activity of leaf extracts of these two plants were evaluated against different plant and human pathogenic fungi including *Candida* and dermatophytes by poisoned food technique and agar-well diffusion method. Moderate to strong antifungal activity against all tested organisms was noted.

Keywords : *Cassia tora, Cassia sophera,* Antifungal, *Candida,* Dermatophytes,

INTRODUCTION

Mycosis is the fungal infection in human caused either due to their weakened immune system or due to predisposing factors like prolonged chamotherapy. Patients with diabetes mellitus and those infected with HIV I and HIV 2 are most susceptible to an opportunistic mycosis called candidiasis. If untreated it may persist for long period. Cutaneous fungal infections called dermatophytoses pose a therapeutic problem and include invasive hair, nail and skin infections. They are commonly called as ring worm or tinea (Dr Arthur Disalvo). Drugs of choice for candidiasis include clotrimazole, fluconazole or nistation (Prabhakar *et al.,* 2008). But due to increase in instances of durg resistance as well as due to undesirable side effects of synthetic drugs, there is a need to develop and shift to herbal remedies.

There is evidence of herbs having been used in the treatment of diseases and for revitalizing body systems in ancient medical systems of India, Egypt, China, Greece and Rome.

Phytochemical analysis revealed antifungal components like chrysophanic acid-9-anthrone in seeds of *C. tora* (Acharya and Chaterjee 1975) and ascrobic acid dehydroascorbic acid and Beta-sistosterol in *C. sophera* seeds (Ahmad Bilal *et al.,* 2005). These substances were recommended for primary healthcare to treat ringworm and skin diseases. (Phongpacichit *et al.,* 2004). The present study is on the *in vitro* antifungal activity of these two wild herbs. *Cassia tora* and *C. sophera* which grow in warangal and other parts of India as weeds. according to Ayurveda the leaves and seeds of *Cassia tora* are laxative, antiperiodic, antihelmintic liver tonic, cardiotonic and expectorant. The leaves were used in treating leprosy, ring worm, constipation etc. (Deore *et al.,* 2009) *Cassia sophera* was used in respiratory disorders, including asthma and bronchitis (Nagore *et al.,*). Though antifungal activity of seed extracts of *Cassia tora* has been reported (Ahmad *et al.,*) leaf extracts of these plants have not been screened for their antifungal activity against various human and phytopathogenic fungi. In the present study preliminary chemical tests of leaf extracts suggested the presence of antifungal substances. Further isolation, characterization and quantification of these bioactive compounds is under way for a novel phytotherapeutic drug development process.

MATERIAL AND METHODS

Fresh leaves of *Cassia tora* and *C. sophera* growing in their natural conditions were collected, washed with tap water, shade dried and finely powdered in a mixer. Leaf extracts were prepared by cold extraction method and also with soxhlet apparatus, Besides water different organic solvents like methods, butanol, ethanol, ethanol, acetone, propanol, chloroform and petroleum ether were used. Collected extracts were concentrated by evaporation, *i.e.,* exposing them to steam from hot water.

For antifungal activity, plates containing solidified potato dextrose agar medium were inoculated with clinical isolates of *Candida* albicans. Plates with Sabourand's agar were inoculated with clinical isolates of *Trichophyton mentagraphitis* and *Microsporum gypsum*. Filamentous fungi like *Aspergillus, Penicillium, Fusarium, Alternaria, Drechsclera* and *Curvularia* were isolated from infected plants and their spore suspensions were inoculated onto PDA plates.

For agar well-diffusion method (Packlalakshmi 2009) wells of 0.5 cm diameter were made in the inoculated plates

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with the help of cork borer. 100 microliters of each extract was introduced into the wells with the help of micropipette, various solvents were used as control. Fluconazole was used as standard antifungal agent, plates were incubated at 28°C for 72 hrs for *Candida* and dermatophytes and for 7 days for filamentous fungi. Inhibition zones were measured.

To detect antifungal activity of aqueous extracts of *C. tora* leaves, poisoned food technique (Reddy, 2008) was used. Boiled aqueous extract filtered through double layered muslim cloth was used as stock. From this different dilutions of 25%, 75% were made with sterile distilled water. Each concentration of the extract was mixed aseptically with sterile PDA medium (1: 1 v/v) and poured in petriplates. The pathogenic fungal mycelial discs of 1 cm diameter, using sterile cork borer were taken out of 6-7 days old culture maintained on PDA medium and inoculated on the poisoned medium in each petriplate. A set of control was also kept. All plates were incubated for 9 days at 28°C diameter of the colony growth was measured in each petriplate and the percentage of inhibition of the fungal growth was calculated using the formula, Percentage of inhibition = {(C - T) 100/C}, C = diameter of colony growth in control. T = diameter of colony growth in test.

RESULTS AND DISCUSSION

The leaf extracts of *C. tora* and *C. sophera* inhibited the growth of test fungi. While *Candida* was most inhibited *Penicillium* and *Fusarium* species were inhibited by all solvent extracts except acetone extract of *C. tora*. Methanol extracts of *C. tora* showed maximum inhibition and petroleum ether extracts showed least inhibition of the test fungi (Table 1 and Fig).

Aqueous extracts of *C. tora* inhibited all test fungi and the percentage of inhibition increased with increase in the concentration of leaf extract. The average growth diameter of the colony and percentage inhibition of the colony is presented in Table 2 and Fig.

C. sophira extracts showed moderate activity when compared to that of *C. tora*. Methanol extracts inhibited all the fungi used. Inhibitory activity with chloroform and petroleum ether extracts appeared to be weak. *Aspergillus* did not respond to many extracts. Dermatophytes tested were inhibited by methanolic leaf extracts of both plants. (Table 3 and Fig).

Above results clearly depict the efficacy of these two plants against human mycosis and plant fungal diseases. Recently there is a great demand for antifungal agents for the treatment of mycosis. Development of drug resistance in fungi and increased therapeutic failures in allopathic medicine leading towards search of alternate herbal medicines with potential antifungal activities, has necessitated plant-based treatment as an ecofriendly approach.

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Table 1 : Diameter of inhibition zones of *C. tora* extracts in cms.

Fungi used	Acetone extract	Ethanol extract	Methanol extract	Propanol extract	Butanol extract	Chloroform extract	Petroleum extract	Fluconazole extract
Aspergillus	0.5	1.5	1.0	1.5	0.5	0.5	0.5	1.5
Penicillium	0.4	0.5	0.5	0.4	0.25	0.5	0.6	1.0
Fusarium	0.7	0.5	1.0	0.5	0.25	0.2	0.2	0.7
Alternaria	1.0	0.5	0.5	0.25	0.2	0.6	ND	0.3
Drechslera	1.0	ND	0.5	0.2	0.2	0.5	ND	3.0
Curvularia	0.5	ND	0.25	0.2	0.2	ND	0.5	2.0
Candida albicans	0.5	2.0	0.5	1.5	0.5	0.5	1.0	0.5
Microsporum gypsum	NT	NT	0.8	NT	NT	NT	NT	0.6
Trichosporum mentagraphitis	NT	NT	0.5	NT	NT	NT	NT	0.5

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Table 2 : Diameter of inhibition zones of *C. sophera* extracts in cms.

Fungi used	Acetone extract	Ethanol extract	Methanol extract	Propanol extract	Butanol extract	Chloroform extract	Petroleum ether
Aspergillus	ND	ND	0.5	ND	ND	ND	ND
Penicillium	0.5	1.0	0.5	ND	ND	0.4	ND
Fusarium	0.5	0.6	0.4	0.5	0.5	ND	ND
Alternaria	0.6	0.4	0.5	0.5	0.7	0.2	0.2
Drechslera	0.5	0.2	1.0	0.4	0.3	ND	ND
Curvularia	0.5	0.6	2.0	0.4	1.0	ND	0.5
Candida albicans	0.7	0.4	0.8	0.5	0.4	0.3	0.5
Microsporum gypsum	NT	NT	0.6	NT	NT	NT	NT
Trichosporum mentagraphitis	NT	NT	0.6	NT	NT	NT	NT

ND = Not detected. NT=Not tested.

Table 3 : Percentage inhibition with aqueous extracts of *C. tora.*

Fungi used	Plate	Percentage concentration	Diameter of colony in cms	% ihhibition
Aspergillus	Plant extract	25	2.0	50
		75	1.5	62.5
		100	1.0	75
	Control		4.0	
Penicillium	Plant extract	25	1.5	57
		75	0.75	78
		100	0.5	85
	Control		3.5	
Fusariium	Plant extract	25	1.5	57
		75	1.0	71
		100	0.8	77
	Control		3.5	
Alternaria	Plant extract	25	1.5	50
		75	1.2	60
		100	0.5	83
	Control		3.0	
Drechsclera	Plant extract	25	0.8	60
		75	0.6	70
		100	0.6	70
	Control		2.0	
Curvularia	Plant extract	25	2.5	10.7
		75	2.0	28.5
		100	1.0	64.28
	Control		2.8	

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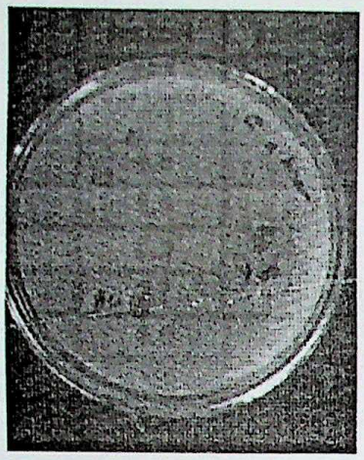
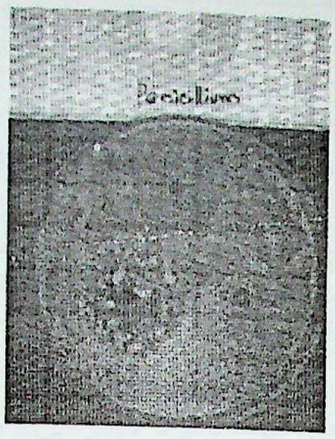
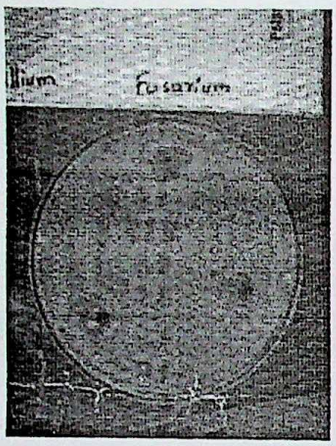
Fungi Inhibited by *Cassia* extracts



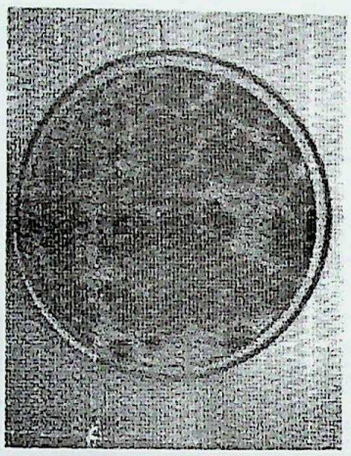
Candida albicans



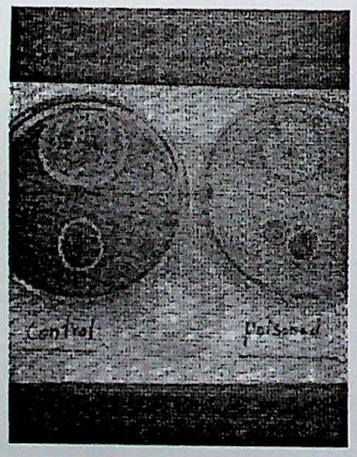
Penicillium



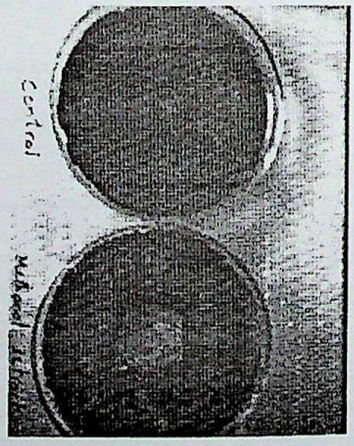
Fusarium



Drechsclera



Poison food technique



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Antibacterial Activity of *Cucumis melo* L. (Syn : *Cucumis pubescens* Willd.) and *Phyllanthus maderaspatensis* L.

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Antibacterial screening of ethanol and chloroform extracts on two medicinal plants of fruits of *Cucumis melo* L. (Syn : *Cucumis pubescens* Willd.) and whole plants of *Phyllanthus maderaspatensis* L. was performed in both disc and well diffusion method against four human skin pathogenic bacteria. Out of this two extracts the ethanol extracts of both the plants in disc diffusion method showed maximum and broad - spectrum antibacterial activity against all four pathogens *Escherichia coli, Pseudomonas aeruginosa, Salmonella* sp. and *Staphylococcus aureus.*

Keywords : Medicinal plants, Antibacterial activity, Ethanol and Chloroform extracts, *Escherichia coli, Pseudomonas aeruginosa, Cucumis melo* L. (Syn : *Cucumis pubescens* Willd.), *Phyllanthus maderaspatensis* L.

INTRODUCTION

In recent years, there has been a growing interest to evaluate plant possessing antimicrobial activity for various diseases. A number of studies have been reported dealing with antimicrobial screening of extracts of medicinal plants. Plant derived drugs have become a popular alternative medicine in developing countries. The synthetic antifungal and antibacterial drugs widely used at present sometimes cause toxicity and averse drug reactions. Furthermore herbal medicine and supplementation are considered less toxic than synthetic drugs. However, folk medicines have not been studied extensively. *Cucumis melo* L. (Syn: *C. pubescens* Willd.) and *Phyllanthus maderaspatensis* L. are used to treat various diseases in traditional system of medicine. The study plants are widely used as the area of fever, diarrhoea, dysentery and skin disease. However, very little work has been done to evaluate for antimicrobial properties of plants. The present study was undertaken to investigate antibacterial activity of ethanol extract and chloroform extract of *Cucumis melo* L. (Syn : *C. pubescens* Willd.) and *Phyllanthus maderaspatensis* L. against various strains of bacteria.

MATERIAL AND METHODS

Cucumis melo L. (Syn : *Cucumis pubescens* Willd.) and *Phyllanthus maderaspatensis* L. are the materials of the present investigation. Fresh fruits of *C. melo* L. (Syn : *C. pubescens* Willd.) are collected from Vilamuthur village, Perambalur, Tamil Nadu, and that of *P. maderaspatensis* L. from Thanthai Hans Roever College, Perambalur, Tamil Nadu, India. Field visits were undertaken to different localities of villages. Information about the plant possessing wounds, dental curing activities and skin disease were collected from native medicine mean.

The study plants were identified with the help of available Indian literature and the identified plants were verified with the help of Botanical Survey of India, TNAU, Coimbatore, India and also in Rapinet Herbarium, St. Joseph's College (Autonomous), Tiruchirapalli, Tamil Nadu, India. The botanical studies were collected from available Indian literatures (Kirtikar and Basu, 1999). The ecological studies of the plants were collected from some Indian literature (Anonymous, 1952). The phytochemical constituents of the study plants were collected from available Indian literatures (Rastogi and Mahrota, 1993; Trease and Evans, 1989). Medicinal uses of the plants were collected from the available Indian literatures on medicinal plants (Nadkarni, 1976; Kirtikar and Basu, 1999; Kaushik, 2003; Kaushik and Chauhan, 2009; Kaushik and Dhiman, 2000). Herbarium specimen are deposited in Botanical Survey of India, TNAU, Coimbatore, Tamil Nadu, India and also in the Rapinet Herbarium, St. Joseph's College (Autonomous), Tiruchirapalli, Tamil Nadu, India. The plant herbarium numbers obtained *C. melo* L. (Syn : *C. pubescens* Willd.) was Voucher No. RTH-29026 and for *P. maderasatensis* L. Voucher No. RHT-43071.

Preparation of plant powder and extracts : The collected fruits of *C. melo* L. (Syn : *C. pubescens* Willd.) and whole plants of *P. maderaspatensis* L. was shade dried at room temperature for 3 days and sundried for 3 days and then milled into coarse powder by a mechanical grinder (Harbone, 1988). 50 g of leaf

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and fruit powder of the two plants was taken in a 250 ml conical flask and 100 ml of ethanol (85%) was added. The ethanol-leaf and fruit powder mixtures were kept at room temperature for 48 hours. After 48 hours, the extract of each plant was filtered through Whatmann No. 1 filter paper to exclude the leaf and fruit powder. Then each filterate was kept in a beaker on a water bath at 45°C until the solvent got evaporated. The greasy final material (crude extract) obtained from each plant was transferred to screw cap bottles and stored under refrigerated conditions till use. Fruits and leaves were ground in mortar and pestles. The ground pulp was then placed in a percolator and 20 ml of chloroform was added to stand for 30 minutes. The filtrate thus obtained was the chloroform extract.

Microorganisms used : *Escherichia coli* (ATCC 9837), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella* sps, *Staphylococcus aureus* (ACTT 6538) were obtained from Doctor's Diagnostic Centre (DDC), Trichy.

Discs of 5 mm in diameter from a sheet of filter paper were punched and placed in Petridishes allowing a distance of 2 - 4 mm between each disc and sterilized in a hot air over at 160°C for 1 hour. After allowing the disc to cool, and 100 mg/ml of each test solution were added on to each disc and than the discs were dried at 37°C in an incubator for one hour (Cheesbrough, 1999). Nutrient agar Petriplates were swabbed with broth culture of each test bacteria in separate plate by sterile swab. Over this, prepared antimicrobial discs were placed under aseptic conditions. Discs of each extract were placed in triangles. Control set with standard antibiotic Oxacillin, Piperacillin and Rifamicin (10 mg/disc) were simultaneously maintained. The plates were than incubated at 37°C for 24 hrs and the zone of inhibition (IZ) was measured and recorded.

RESULTS AND DISCUSSION

Ethanolic and chloroform extracts of *C. melo* L. (Syn: *C. pubescens* Willd.) were selected at a concentration of 100 mg/ml. The ethanolic extracts showed maximum antibacterial activity in disc diffusion method against *Pseudomonas aeruginosa* (11.2 mm I.Z), *Staphylococcus aureus* (8.3 mm I.Z) and *Escherichia coli* (11 mm I.Z) and minimum zone of inhibition against *Salmonella* sp. (6.3 mm I.Z) (Table 1). The ethanolic extracts of *C. melo* L. (Syn: *C. pubescens* Willd.) showed maximum antibacterial activity in well diffusion method against *Escherichia coli* (11mm I.Z), *Salmonella* sp (8.3 mm I.Z). and *Staphylococcus aureus* (8.3mm I.Z) and minimum zone of inhibition against *Pseudomonas aeruginosa* (7.6 mm (I.Z) (Table 2).

Chloroform extracts of *C. melo* L. (Syn: *C. pubescens* Willd.) showed maximum antibacterial activity in disc diffusion method against *Escherichia coli* (7.6mm I.Z) and *Staphylococcus aureus* (6.6 mm I.Z) and no zone of inhibition against *Pseudomonas aeruginosa* (Table 3). Chloroform extracts of *C. melo* L. (Syn: *C. pubescens* Willd.) showed maximum antibacterial activity in well diffusion method against *Escherichia coli* (8 mm I.Z) and *Staphylococcus aureus* (7mm I.Z) and no zone of inhibition against *Pseudomonas aeruginosa* (Table 4). Ethanolic and chloroform extracts *P. maderaspatensis* L. were selected at a concentration 100 mg/ml. Ethanol extract of *P. maderaspatensis* L. showed maximum antibacterial activity in disc diffusion method against *Pseudomonas aeruginosa* (9.3mm I.Z), *Escherichai coli* (7.8mm I.Z) and *Staphylococcus aureus* (7.3 mm I.Z) and minimum zone of inhibition against *Salmonella* sp. (4.6 mm I.Z) (Table 1). The ethanolic extracts of *P. maderaspatensis* L. showed maximum antibacterial activity in well diffusion method against *Salmonella* sp., (10.3mm I.Z) *Pseudomonas aeruginos* (9.3mm I.Z) and *Staphylococcus aureus* (8 mm I.Z) and minimum zone of inhibition against *Escherichia coli* (6.6mm I.Z) (Table 2). The chloroform extract of *P. maderasptensis* L. showed maximum antibacterial activity in disc diffusion method against *Escherichia coli* (9.6mm I.Z) and *Staphylococcus aureus* (8.6mm I.Z) and no zone of inhibition against *Pseudomonas aeruginosa* (Table 3). Chloroform extract of *P. maderaspatensis* L. showed maximum antibacterial activity in *Staphylococcus auresu* (10 mm I.Z) and *Escherichia coli* (9 mm I.Z) and no zone of inhibition against *Pseudomonas aeruginosa* (Table 4).

The effects of zone of inhibition were compared with commercially available antibiotics such a Oxacillin, Piperacillin and Rifamcin. Ethanol and chloroform extracts of *C. melo* L. (Syn: *C. pubescens* Willd.) and *P. maderaspatensis* L. showed more pronounced zone of inhibition against selected microorganisms than standard drugs (Table 1 - 4).

The phytochemicals like phenolics can cause membrane disruption, from complex with cell wall, inactivate enzymes and cause substrate deprivation (King and Tempesta, 1994). Alkaloids can interchelate into cell wall and DNA. (De Leo *et al.*, 2004) also given some new phenolic compounds, 1-galloyl-β-glucopyranosyl β-D-galactopyranoside, 2-methoxy-5-(1', 2', 3'-trihydroprophyl)-phenyl-1-0-(6" galloyl)-β-D-glucopyranoside, methoxy, 5-hydroxymethyl-phenyl-1-0 (6" galloly) β-D-pyranoside, together with the known compounds benzoyl-0-gathyl-β-D-pyranose, myrciaphenone, kaemplfero-3-0-(6"-β-D-glucopyranoside, quercetin 3-0 (6"galloyl)-β-pyranosie, Vomifoliol-9-0-β-D-apiofuranosyl-(1-6)-glucopyranoside,2,3-dihydrobenzofuran-2-(4' hydroxy3;Oxyphenyl)-3α-O rhamnopyranosylooxy methyl-7-methoxy and benzyl-O-α L-rhamnopyranosyl (1-6)-β-D pyranoside were isolated from the leaves of *Baseonema acuminatum*. The antimicrobial properties, of two clerodane diterponoids (compounds A and B) and spinasterol (compound C), clerodane diterpenoids are known to possess anti-tumoral, antibacterial and antifungal properties (Biswanath *et al.*, 2005; Murthy *et al.*, 2005; Urzua *et al.*, 2006). Compound A was more effective on Gram (+) bacteria than Gram (–) and with MICs lower than that of reference antibacterial Gentamicin. This observation is in agreement with data mentioned by some other authors as far as clerodane diterpenoids are concerned (Murth *et al.*, 2005; Urzua *et al.*, 2006; Tojo *et al.*, 1999; Teponno *et al.*, 2006; Rezende *et al.*, 2000).

These phytochemicals may be responsible for antibacterial activity angainst selected pathogens. It is

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Table 1 : **Antibacterial activity of ethanol extract of fruits of *Cucumis melo* L. (Syn : *C. pubescens* Willd.) and whole plants of *Phyllanthus maderaspatensis* L. by disc diffusion method.**

S.No.	Organism	Zone of inhibition (mm) ethanol extract (100 mg/ml)		Standard drug for 10 mg (mm)
		C. melo L. (Syn : *C. pubescens* Willd.)	*P. maderaspatensis* L.	
1.	*Pseudomonas aeruginosa*	12	9.3	11
2.	*Staphylococcus aureus*	8.3	7.3	7.6
3.	*Escherichia coli*	11	7.8	10.6
4.	*Salmonella* sp.	6.3	4.6	13

Table 2 : **Antibacterial activity of ethanol extract of fruits of *Cucumis melo* L. (Syn : *C. pubescens* Willd.) and whole plants of *P. maderaspatensis* L. by well diffusion method.**

S.No.	Organism	Zone of inhibition (mm) ethanol extract (100 mg/ml)		Standard drug for 10 mg (mm)
		C. melo L. (Syn : *C. pubescens* Willd.)	*P. maderaspatensis* L.	
1.	*Escherichia coli*	11.3	6.6	10.3
2.	*Pseudomonas aeruginosa*	7.6	9.3	13
3.	*Salmonella* sp.	8.3	10.3	14
4.	*Staphylococcus aureus*	8.3	8	5.3

Table 3 : **Antibacterial activity of chloroform extract of fruits of *Cucumis melo* L. (Syn : *C. pubescens* Willd.) and whole plants of *Phyllanthus maderaspatensis* L. by well diffusion method.**

S.No.	Organism	Zone of inhibition (mm) ethanol extract (100 mg/ml)		Standard drug for 10 mg (mm)
		C. melo L. (Syn : *C. pubescens* Willd.)	*P. maderaspatensis* L.	
1.	*Escherichia coli*	7.6	9.6	8
2.	*Pseudomonas aeruginosa*	-	-	-
3.	*Staphylococcus aureus*	6.6	8.6	5.6

Table 4 : **Antibacterial activity of Chloroform extract of fruits of *Cucumis melo* L. (Syn : *C. pubescens* Willd.) and whole plants of *Phyllanthus maderaspatensis* L. by well diffusion method.**

S.No.	Organism	Zone of inhibition (mm) ethanol extract (100 mg/ml)		Standard drug for 10 mg (mm)
		C. melo L. (Syn : *C. pubescens* Willd.)	*P. maderaspatensis* L.	
1.	*Escherichia coli*	8	9	8.6
2.	*Pseudomonas aeruginosa*	-	-	-
3.	*Staphylococcus aureus*	7	10	6

concluded that antibacterial activity of *C. melo* L. (Syn: *C. pubescens* Willd.) and *Phyllanthus maderaspatensis* L. and its active phytochemical compounds can be helpful in the therapeutic treatment for selected pathogenic epithelization. The study plants have not yet been tested for antimicrobial activity against human skin pathogens. Hence the present study was undertaken to investigate their antibacterial activity against human skin pathogens.

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Antibacterial Assay - A New Tool for Taxonomic Verification of *Datura* (Solanaceae)

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Datura remains a riddle to the taxonomist due to their morphological variations in wide-ranging geographical distribution. Morphologically, *D. ferox* (fruits with long spine), *D. stramonium* (fruit with slender spine of equal size and dehisce regularly) and *D. innoxia* (persistent and prominent calyx) have very distinct characters hence less of controversy to delineate them. Where as *D. fastuosa, D. metel* and *D. metel* var. *fastuosa* pose difficulties in identification and differentiation thus quite often they are misidentified or treated as synonyms. The present investigation was aimed at delineating the taxonomic evidences of six species of *Datura* based on their antibacterial potency. The ethanolic and aqueous extracts of selected species of *Datura* were screened for their antibacterial activity against ten bacterial strains (*Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella typhi, Proteus mirabilis, Proteus vulgaris, Klebsiella pneumonia, Enterobacter aerogenes, Serratia marcescens* and *Pseudomonas aeroginosa*). The results showed that *D. fastuosa* and *D. metel* var. *fastuosa* have greater potency against seven test bacteria while, *D. metel* showed minimum inhibitory activity. *D. ferox* and *D. stramonium* do not have antibacterial activity. Thus the study of antibacterial assay could efficiently serve the purpose of delineating species.

INTRODUCTION

Datura is well known in both Old and New World by its effect as hallucinogen from the earliest time (Hawken *et al.*, 2009). Besides its hallucinogen activity, *Datura* reported to have antimicrobial activity (Rajesh & Sharma, 2002; Kaushik & Goyal, 2008), to treat asthma, tuberculosis, Parkinson's disease (Purohit & Vyas, 2004; Khare, 2007) and wound healing effect in albino rats (Shanmuga *et al.*, 2002). Due to their therapeutic uses (domesticated at herbal garden) and its potency to adapt and grow at varied eco-climatic regions of the world, the species level delimitation of the genus is found to be complex.

Plant taxonomy, an oldest branch in botany/plant biology/plant science is enriched by the development in science and technology. Thus any new progress emerged in the past starting with anatomy, embryology, karyology to molecular techniques (PCR, DNA fingerprinting, gene sequencing) and computer applications (numeric taxonomy) have contributed tremendously for classification and identification of plant taxa. In line with the above, though the antimicrobial assay is much older than many of the recent molecular adventure, it not being used to solve taxonomic misunderstanding. Hence, a novel initiative is taken to apply the information of microbial assay to delineate the taxa at species level. The genus *Datura*, is the much problematic and controversial genus in the family Solanaceae. The fundamental logic behind this experiment is that the antimicrobial activity of plants are due the various secondary metabolites produced by them and in term the secondary metabolites are expression of the genetic make up of plants themselves. Hence, any change in the activity of plant within the genus against test organism is direct indication of genetic difference within themselves. The microbes are highly sensitive to these compounds which could profoundly assist and confirm the difference with in a genus.

MATERIAL AND METHODS

The different species of *Datura* (*D. ferox* L., *D. stramonium* L., *D. innoxia* Mill., *D. fastuosa* L., *D. metel* L. and *D. metel* L. var. *fastuosa* (L.) Saff.) were collected from various locations in Tamil Nadu, India. The voucher specimens have been deposited in the Herbarium, Department of Plant Biology and Plant Biotechnology, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India.

Extraction procedure and bacteria used: The collected plant materials (leaves) were washed in tap water followed by sterilized distilled water, shade dried for 15 days and then powdered. The powders were further subjected for different extraction protocols. The aqueous and ethanolic solvents were used in this study. About 3 g of powder of each plant was grinded with 20 ml of distilled water. The solution was filtered through muslin cloth and the filtrate was collected in fresh sterilized bottles. For ethanolic extract, 10 g of shade-dried

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powder was thoroughly mixed with 60 ml of ethanol in brown bottle. The mixture was placed at room temperature for 1 week. The solution was filtered, thus obtained filtrate was concentrated by complete evaporation of solvent at room temperature to yield the pure extract and quantify the yield. All the extracts were stored at 4° C until further use.

Ten bacterial strains (*Staphylococcus aureus, Bacillus cereus* (Gram-positive) and *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Proteus mirabilis, Proteus vulgaris, Serratia marcescens, Enterobacter aerogenes* and *Klebsiella pneumoniae* (Gram-negative) were used in this investigation. All the bacterial cultures were obtained from the Doctors Diagnostic Centre, Tiruchirappalli, Tamil Nadu. These bacterial cultures were maintained in nutrient agar slants in refrigerator. The bacteria was reactivated prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37°C.

Screening for Antibacterial assay : The antibacterial activity was assessed by disc diffusion method (Maruzella & Henry, 1958). The sterile nutrient agar medium (20 ml) was poured into sterile petriplates. 0.1 ml of overnight bacterial suspension (10^8 CFU/ml) of each test bacteria were spread over nutrient agar plates. ÿhe sterile discs (6 mm in diameter) were impregnated with 10 µl of plant extract (30 µg/disc) placed on top of agar plates. The positive controls were also maintained using commercial antibiotic chloramphenicol (30 µg/disc). All the plates were incubated at 37°C for 24 hours. The diameter of growth inhibition zone of each petriplate was measured and the results were tabulated. All the experiments were done in triplicate and the mean and standard deviation was calculated.

RESULTS AND DISCUSSION

Morphologically the species of *Datura* can be differentiated following the key

1. Fruit dehisce regularly 2
1. Fruit dehisce irregularly 3
2. Spine on fruit - unequal in size, more than 2 cm long at apex.....*ferox*
2. Spine on fruit-equal in size, 1 cm long ..... *stramonium*
3. Stem-hirsute, corolla lobe 10, calyx in fruit > 2 cm wide, spine on fruit >1 cm ..... *innoxia*
3. Stem-nearly glabrous, corolla lobe 5, clayx in fruit < 1 cm wide, spine on fruit < 1 cm 4
4. Fruit-oval, corolla lobe circinate .....*metel*
4. Fruit other than oval, corolla lobe not circinate 5
5. Corolla-single layer, colour white, fruit-globular .....*fastuosa*
6. Corolla - 2 - 3 layer, colour purple, fruit-oblong .....*metel* var. *fastuosa.*

There is much confusion in differentiating *D. fastuosa* from *D. metel*, hence Kumari (2004) merged them into single species based on the morphological features. However, the present authors were able to identify a few morphological characters to differentiate the same such as the globular shape of fruit in *D. fastuosa* and oval in *D. metel*. Besides, the corolla lobe apex in *D. metel* is circinate while in *D. fastuosa* it is not so, in addition the colour of corolla is pale yellowish in budding stage which turn to white in maturity in *D. fastuosa* where as it is purple throughout in *D. metel*.

In order to authenticate and to confirm the identity of above findings ethanolic and aqueous crude extracts of the selected plants (different species of *Datura*) were tested for antibacterial activity against 10 bacterial strains and the results are tabulated (Table 1). Antimicrobial activity of plants pertains to the potency of plants for synthesis of secondary metabolites which is the expression of the genetic make up of plants. Hence, the assay of this activity is used to clarify the doubt in the genus *Datura*. There are a few investigations done to document the potency of *Datura* species against microbial activity. But in the present study, this antibacterial assay was used as a character to differentiate the confusing taxa *D. metel* from *D. fastuosa*, since other species are easily separated with the help of morphological features.

Ethanolic and aqueous extracts of root and stem bark of *D. stramonium* inhibited many organisms (Obi *et al.*, 2003). *Pseudomonas aeruginosa* was found to resist the ethanolic extract of *D. stramonium* (Obi *et al.*, 2002; Eftekhar *et al.*, 2005) which is also confirmed from the present study but seem to contratic the report of Bayoud *et al.* (2007). Herbal preparation of *D. stromanium* with cow urine showed higher sensitivity of *Staphylococcus aureus* (Yadave *et al.*, 2008). Methanolic leaf extract of *D. innoxia* was found to be active against *E. coli, Salmonella typhi, Bacillus cereus* and *Staphylococcus aureus* (Kaushik & Goyal, 2008). Similar results have been observed in aqueous and ethanolic extracts of *D. alba* showed high inhibitory activity against *Staphylococcus* sp., *Klebsiella* sp., *E. coli, Pseudomonas* sp. and *Salmonella* sp. (Shanmuga *et al.*, 2002).

The over all results from this study highlights that the antimicrobial assay of the confusing taxon *D. metel* var. *fastuosa* (*Datura* with double petal that normally refer synonym to *D. metel*) could be temporarily rectified. *D. metel* var. *fastuosa* is very closer to *Datura fastuosa* in its antibacterial assay and distant from *D. metel* (Table 1). This conclusion is also affirmed by the petal apex not being circinate in both *D. fastuosa* and *D. metel* var. *fastuosa*. Most probably *D. metel* var. *fastuosa* being an ornamental must have been the result of crossing of *D. fastuosa* and *D. metel*. Relatively *D. metel* shows very minimum antibacterial activity compared to *D. fastuosa* and *D. metel* var. *fastuosa*. Aqueous extract of *D. metel* has inhibited the growth of *Klebsiella pneumoniae, Enterobactor aerogenes* and *Serratia marcescens* only but in contrast *D. fastuosa* inhibited almost all tested bacteria. Hence, based on a few morphological features and the antibacterial assay, it is concluded that *D. fastuosa* and *D. metel* var. *fastuosa* are clearly distinct from *D. metel*. The present study also intuited that the compression of *D. metel* var. *fastuosa* and *Datura fastuosa* as *D. metel* needs other investigation such as molecular or embryology to authenticate the present report.

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Table 1 : Antibacterial assay of *Datura* species against test pathogens (diameters in cm with SD).

Plant Name	Solvents	*Staphylococcus aureus*	*Bacillus cereus*	*Escherichia coli*	*Salmonella typhi*	*Proteus mirabilis*	*Proteus vulgaris*	*Klebsiella pneumoniae*	*Enterobactor aerogenes*	*Pseudomonas aeruginosa*	*Serratia marcescens*
Datura ferox	Aqueous	—	—	—	—	—	—	—	—	—	—
	Ethanol	—	—	—	—	—	—	—	—	—	
Datura stramonium	Aqueous	—	—	—	—	—	—	—	—	—	—
	Ethanol	—	—	0.8 0.08	—	—	—	—	—	—	—
Datura innoxia	Aqueous	1.2 ± 0.00	1.3 ± 0.00	—	1.4 ± 0.00	1.7 ± 0.00	—	1.1 ± 0.35	1.2 ± 0.00	—	0.8 ± 0.05
	Ethanol	1.88 ± 0.16	1.66 ± 0.28	0.76 ± 0.05	1.75 ± 0.42	0.76 ± 0.11	2.3 ± 0.16	2.18 ± 0.35	—	—	—
Datura fastuosa	Aqueous	1.4 ± 0.1	—	—	1.43 ± 0.11	2.16 ± 0.15	1.76 ± 0.21	1.86 ± 0.05	1.5 ± 0.26	—	—
	Ethanol	1.8 ± 0.00	1.86 ± 0.11	—	1.7 ± 0.11	—	1.7 ± 0.1	1.9 ± 0.17	1.8 ± 0.00	—	—
Datura metel	Aqueous	—	—	—	—	—	—	0.9 0.00	1.16 0.05	—	0.9 0.05
	Ethanol	—	1.16 ± 0.46	—	0.9 ± 0.1	0.9 ± 0.00	—	1.2 ± 0.05	1.1 ± 0.11	0.9 ± 0.11	—
D. metel var. *fastuosa*	Aqueous	1.3 ± 0.00	1.4 ± 0.1	1.66 ± 0.05	1.2 ± 0.05	1.7 ± 0.00	—	1.56 ± 0.05	1.6 ± 0.05	—	—
	Ethanol	1.1 ± 0.15	1.7 ± 0.1	1.7 ± 0.05	1.4 ± 0.00	1.7 ± 0.1	—	1.6 ± 0.00	1.7 ± 0.00	—	—
Chloramphenicol	Control	2.4 ± 0.00	2.4 ± 0.00	3.0 ± 0.00	2.8 ± 0.00	1.0 ± 0.00	3.4 ± 0.00	4.0 ± 0.0	2.4 ± 0.00	—	3.0 ± 0.00

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Antifungal Activity of Semisynthetic Derivatives of Embelin (*Embelia ribes*)

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Substituted benzisoxazole-4,7- diones, (6-hydroxyl-3-aryl-5-undecyl-1-2-benzisoxazole-4,7-diones), phenazines (3-aryl-4-undecyl isoxazole [5,4-a]-Phenazines), acetoxyl-1,2- benzisoxazole diones (6-acetoxyl-3-aryl-5 undecyl-1,2-benzisoxazole-4,7-diones and Benzoxadiazines (N-Acetyl-2-aryl-6,7- diacetoxy-8-undecyl benzoxadiazines) synthesized from *Embelin* were active, effective against *Curvularia lunata* and *Fusarium oxysporum.*

Keywords : *Embelia ribes,* Fungicidal activity, Benzene extract, *Curvularia lunata* and *Fusarium oxysporum.*

INTRODUCTION

The dried fruits of *Embelia ribes* (Myrsinaceae) is most widely used in tradition herbal medicine in India. The fruits of *Embelia ribes* reported to contain mainly benzoquinone derivatives such as Embelin and vilangin. The dried fruit has been used in India since ancient times as anthelmintic. It is also reported as a health tonic and prevents aging. *Embelia ribes* possess astringent (Kumara Swamy *et al.,* 2007). Carminative, stimulant, antioxidant (Joshi *et al.,* 2007). Anti-spermetogenic (Seth *et al.,* 1982. Gupta *et al.,* 1991, Anand *et al.,* 2006), anti-bacterial (Chitraa *et al.,* 2003) and anticancer activity (Nikolovska-Coleska *et al.,* 2004. Seok Ahn *et al.,* 2007).

The plant *Embelia ribes* contains embelin, quercitol, and fatty ingredients; an alkaloid, christembine, a resinoid, tannins and minute quantities of a volatile oil. (Choudhury *et al.,* 2007. Bhandari *et al.,* 2008. Shahid *et al.,* 2009). It is reported to be effective against tepeworm but not against roundworm or hookworm (Jalapure *et al.,* 2007) *Embelin* occurs in golden yellow needles insoluble in water and soluble in alcohol, chloroform and benzene. The dark colored fatty oil is reported to be similar to linseed and rapeseed oil in its properties. Chemical structure of *Embelin* is having quite resemblance with the structure of natural Coenzyme Q 10 (ubiquinones) and the role of this is well defined in various biochemical protective mechanism (Kobaisy *et al.,* 2008).

Antifungal screening of this plant extracts is not studied in detail or if studied is not extended to the different spectrum of fungi and only selective compounds were investigated (Feresin *et al.,* 2003. Latha *et al.,* 2007). Hence in the present study different compounds of *Embelin* were screened for their antifungal activity.

MATERIAL AND METHODS

The berries of *Embelia ribes* are dried and powdered 250 gms of the powdered fruits of *Embelia ribes* was extracted with benzene using a soxhlet extractor for 6 hrs. The extract was evaporated on steam bath. Cold petroleum ether was added to the residue. It was stirred and filtered through buchner funnel under vacuum. The residue was washed with cold petroleum ether. The residue was dried and recrystallized with chloroform to obtain pure Embelin (Kiehlbauch *et al.,* 2000).

Antifungal Activity : Macroscopic culture of *Curvularia lunata* and *Fusarium oxysporum* isolated from stored grains of maize (*Zea mays*) and mustard (*Brassica nigra*) were employed for assay of antifungal activity of semisynthetic derivatives of quinones of *Embelia ribes.* Glass slide humid chamber technique (Anonymous 1947) was employed for assaying antifungal activity. Spore suspension of test fungi were prepared in different concentrations (120, 360, 600 and 840 µg/ml) and put on sterilized glass slides. The glass slides were placed in a Petri plate containing 3 fold filter paper. Sufficient humidity is maintained to avoid surface evaporation. The spore concentration in a test solution was so adjusted to appear 20-30 spores per high power of the microscope (40 x). The Petri dishes thus prepared were incubated at 27-29°C for 8 hrs at the end of incubation period the number of spore germinated were screened. Sterile water in a place of test compound served as a control. At least 200-300 spores were counted for calculation of percentage of spore germination inhibition with the help of the following formula.

$$\text{Percentage of spores Germination inhibition} = \frac{\text{No. of spores germinated in extract}}{\text{No. of spores germination in control}} \times 100$$

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RESULTS AND DISCUSSION

In the present investigations N-Acetyl-2-Aryl-6,7-diacetoxy-8-undecyl benzoxadiazines is highly active against both the fungi, at minimum concentrations (120µg/ml) and the activity was maximum at higher concentration. (600µg/ml and 840µg/ml). 2-methyl substituted benzoxadiazine activity was similar to that of 2-aryl substituted Benzoxadiazines. But remaining substituted Benzoxadiaznes showed moderate activity against both the fungi (Table-1). Similarly Sanjesh *et al.*, 2010. also reported the antifungal activity of *Embelia ribes* plant extracts at lower concentration.

Table-2 reveals that 6-Hydroxy-3-aryl-5-undecyl-1,2-benzisoxazole-4,7-diones were found to be mild in their fungicidal activity. Phenyl substitutes Benzisoxazole was active at 840µg/ml concentration against both the fungi under investigation. While *p*-Cl-C_6H_4 substituted, exhibited marginal fungi toxicity against both the fungi under investigation. Interestingly phenyl benzisoxazole was comparatively more active than *p*-Cl- phenyl substituted compounds.

3-aryl-5-undecyl isoxazole [5, 4-a] Phenazine 4-(6H)-ones were having mild activity against for all concentrations, the chloro phenyl substituted has minimum activity(120µg/ml) and maximum at higher concentrations (840 µg/ml).

The acetoxyl derivatives of phenyl substituted benzisoxazole diones were only mild in their fungicidal activity as spore germination inhibition was significant only at high concentration. Interestingly here also acetoxyl derivative of phenyl benzisoxazole was comparatively active than *p*-Cl phenyl substitand fungi under investigation.

CONCLUSION

It is evident from the present study that the compounds antifungal activity of *Embelin* varied with substitutents on Benzisoxazole and Benzoxadiazins. The Benzoxadiazine compounds were fungi toxic even at lower concentrations.

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Table 1 : Fungicidal activity of benzoxadiazine compounds derived from *Embelin*.

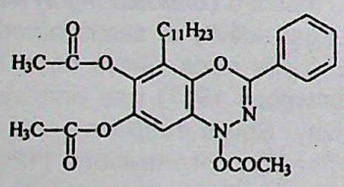
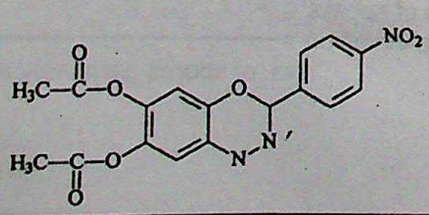
Compound	Concentration µg/ml	Percentage of spore germination	
		C. lunata	*F. oxysporum*
N-Acetyl-2-aryl-6, 7-diacetoxy-8-undecyl-benzoxadiazines	120	85.24	91.28
	360	90.33	94.31
	600	95.00	100.00
	840	100.00	100.00
	120	88.26	90.24
	360	91.00	93.34
	600	95.26	95.28
	840	98.78	99.00
	120	78.62	79.78
	360	81.70	83.36
	600	84.56	87.62
	840	88.76	91.78
	120	65.38	70.56
	360	70.26	74.82
	600	94.34	78.93
	840	82.53	84.56

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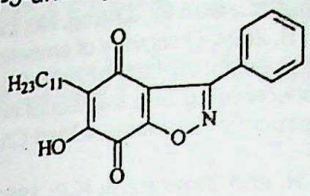
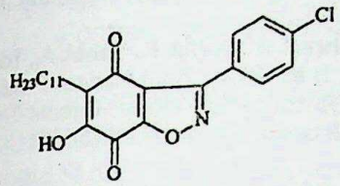
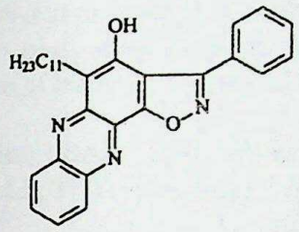
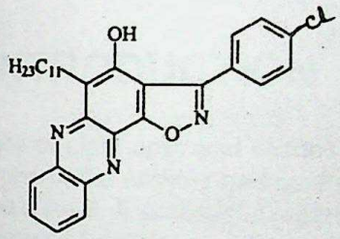
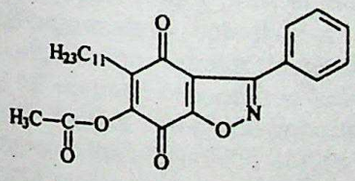
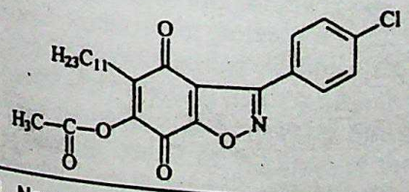
Table 2 : Fungicidal activity of benzisoxazole-4,7-dione compounds derived from *Embelin*.

Compound	Concentration µg/ml	Percentage of spore germination	
		C. lunata	*F. oxysporum*
6Hydroxy-3-aryl-5-undenyl-1,2-benzisoxazole-4,7-diones 	120	--	--
	360	13.59	16.80
	600	59.52	63.33
	840	79.51	89.75
	120	--	--
	360	--	--
	600	14.56	13.74
	840	27.39	28.85
3-Aryl-5-undecylisoxazole [5,4-a] phenazin-4- (6H)-ones 	120	--	--
	360	11.47	11.44
	600	21.94	22.69
	840	42.00	40.00
	120	27.21	25.21
	360	44.54	42.75
	600	62.54	60.31
	840	70.44	70.83
6-Acetoxyl-3-aryl-5-undecyl-1,2-benzoisoxazole-4,7-diones 	120	--	--
	360	34.01	38.21
	600	52.65	49.88
	840	64.32	71.11
	120	--	--
	360	--	--
	600	20.75	21.97
	840	35.73	34.51

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In Vitro Regeneration Response of the Leaflet Explants of ICGS 11 Cultivar of *Arachis hypogaea* L.

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In the present study plant regeneration was achieved from leaflet explants of *Arachis hypogaea* (groundnut) cutivar ICGS-11. The leaflet explants of 1 week old seedlings were stimulated to undergo raft formation on Murashige and Skoogs medium supplemented with auxin and cytokinin hormones. The rafts were then separated and cultured in the erlenmayer flasks containing medium of the same composition. Regeneration was best observed at 4.5 mgL^{-1} BAP with 1.0 mg L^{-1} NAA showing rafts with rooting. Though IAA was also tested but NAA in combination with BAP gave better regeneration. The prominent leaf buds on the raft with rooting were observed at optimal concentrations of 4.5 mgL^{-1} BAP + 0.1 mgL^{-1} NAA+3-5 mgL^{-1} L-Glutamic acid. The present study establishes the optimal hormonal concentrations and importance of amino acids in the regeneration of leaflet explants of ICGS 11 cultivar of *Arachis hypogaea* (groundnut).

Abbreviation : BAP – Benylamino purine, NAA – Napthalene acetic acid, IAA – Indole acetic acid, LGA – Glutamic acid., Tr-Tryptophan, P.A-Phenyl alanine.

INTRODUCTION

Tha capacity of plant cells and tissues to regenerate and propogate is one of the exciting and useful aspects of *in vitro* cell and tissue culture. A successful regeneration mostly depends on the choice of explant and the composition of the medium especially hormones.

Micropropagation offers greater potential for the conservation and large scale multiplication of useful plants and their successful exploitation. Greater demands are for plants which are used for purpose of food and medicine. *Arachiis hypogaea* L (groundnut) is one of the important high protein oil seed crops for more than half of the world's population. It is a native of Brazil and now being grown in tropical and subtropical as well as warm temperate zones of the world. India is the largest producers of groundnut in the world. Within India, Andhra Pradesh is ranked first yielding... 739 Kg / hectare during 2008 - 2009. As it is an oil yielding crop the demand for the cultivation of groundnut is increasing due to increased demand for vegetable oils. This increase in the demand is with higher standards of living and with growing populations thus creating an even increasing gap between supply and demand. However the lack of potential resistant varieties, fluctuation in weather condition and poor agricultural practices are the major hurdles in groundnut production. Rapid development in agriculture is being achieved by plant tissue culture technology. It not only provides suitable medium but also hormones and optimal condition favouring efficient callus induction and regeneration.

In the present study a successful regeneration of the leaflet explants of groundnut was achieved.

MATERIAL AND METHODS

The seeds if *Arachis hypogaea* L were collected from ICRISAT, Patancheru, Hyderabad. Seedlings were raised aseptically by treating the seeds with 70% alcohol for 1 minute followed by a thorough washing with distilled water. They were then surface sterilized with 0.1% (w/v) aqueous Mercuric chloride for 2 - 3 minutes. After washing for 3 - 4 times with sterilized distilled water the seeds were cultured in the sterilized culture vessels on Murashige and Skoogs (1962) basal medium. The pH of the medium was adjusted 5.8 before autoclaving at 121°C for 15 minutes at 105 Kpa pressure. The culture conditions were maintained on a short light regime for 6 hours at approximately 500 lux and temperature was maintained at 25 + 2°C.

From the seedlings the leaflet explants of 0.5 - 1.0 cm length were incoulated on the MS medium. They were placed in such a way so as to allow both the ends of explant to be in contact with the medium.

To the MS Medium with 3% sucrose and 0.6% agar and the hormones *viz.*, Auxin and Cytokinin were added individually and in combinations.

The explants were placed on the culture Murashige and Skoogs medium with 3% Sucrose and various combinations

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In Vitro Regeneration Response of the Leaflet Explants of ICGS 11 Cultivar of *Arachis hypogaea* L

Table 1 : Regeneration response with BAP + NAA.

Hormone concentration BAP + NAA mgL^{-1}	Percentage of calli regenerating + S.E.	Type of response
0.5BAP + 0.5NAA mgL^{-1}	00.00 ± 00.00	.....
1.5BAP + 0.5NAA mgL^{-1}	00.00 ± 00.00	.....
2.5BAP + 0.5NAA mgL^{-1}	00.00 ± 00.00	.....
3.5BAP + 0.5NAA mgL^{-1}	15.00 ± 00.96	Green callus
4.5BAP + 0.5NAA mgL^{-1}	26.00 ± 00.09	Rafts
5.5BAP + 0.5NAA mgL^{-1}	22.00 ± 00.04	Green globular callus
6.5BAP + 0.5NAA mgL^{-1}	15.00 ± 00.67	Green patches on the callus
0.5BAP + 1.0NAA mgL^{-1}	00.00 ± 00.00	.....
1.5BAP + 1.0NAA mgL^{-1}	10.00 ± 00.05	White roots
2.5BAP + 1.0NAA mgL^{-1}	21.00 ± 00.10	Geen nodular structures
3.5BAP + 1.0NAA mgL^{-1}	25.00 ± 00.39	Green globular structures
4.5BAP + 1.0NAA mgL^{-1}	38.00 ± 00.26	Raft with profuse rooting
5.5BAP + 1.0NAA mgL^{-1}	14.00 ± 00.51	Green callus
6.5BAP + 1.0NAA mgL^{-1}	25.00 ± 00.65	Green patches on the callus

Table 2 : Regeneration response with BAP + IAA.

Hormone concentration BAP + IAA mgL^{-1}	Percentage of calli regenerating + S.E.	Type of response
0.5BAP + 0.5IAA mgL^{-1}	00.00 ± 00.00	.....
1.5BAP + 0.5IAA mgL^{-1}	00.00 ± 00.00	.....
2.5BAP + 0.5IAA mgL^{-1}	00.00 ± 00.00	.....
3.5BAP + 0.5IAA mgL^{-1}	10.00 ± 00.66	Friable green callus
4.5BAP + 0.5IAA mgL^{-1}	22.00 ± 00.50	Green globular structures
5.5BAP + 0.5IAA mgL^{-1}	17.00 ± 00.75	Friable green callus
6.5BAP + 0.5IAA mgL^{-1}	08.00 ± 00.85	Friable green callus
0.5BAP + 1.0IAA mgL^{-1}	00.00 ± 00.00	.....
1.5BAP + 1.0IAA mgL^{-1}	00.00 ± 00.00	.....
2.5BAP + 1.0IAA mgL^{-1}	12.00 ± 00.40	Green callus
3.5BAP + 1.0IAA mgL^{-1}	20.00 ± 00.43	Friable green callus
4.5BAP + 1.0IAA mgL^{-1}	36.00 ± 00.36	Small rafts
5.5BAP + 1.0IAA mgL^{-1}	28.00 ± 00.92	Globular green structures
6.5BAP + 1.0IAA mgL^{-1}	20.00 ± 00.84	Friable Green callus

Table 3 : Regeneration response of L-Glutamic acid with optimal concentrations of BAP + NAA.

Hormone concentration BAP + IAA mgL^{-1}	Percentage of calli regenerating + S.E.	Type of response
2.5BAP + 1.0NAA mgL^{-1} + 3.5 L-G.A.	52.00 ± 00.09	Prominent leaf buds on rafts with roots
2.5BAP + 1.0NAA mgL^{-1} + 3.5 Tr	46.00 ± 00.94	Rafts with roots
2.5BAP + 1.0NAA mgL^{-1} + 3.5 P.A.	4.00 ± 00.79	Rafts with roots

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of hormones (auxins and cytokinins). The pH of the medium was adjusted at 5.8 and autoclaved at 121°C for 200 minutes at 15 lbs of pressure. The cultures were maintained at 24 ± 2°C with the photoperiod of 8 hours with illuminescent lights and were subcultured for every 6 weeks.

Optimization of shoot and root regeneration from leaflet derived callus : Freshly induced calli from leaflet were transferred to different regeneration media with varying concentrations of hormones. In the first part of experiment BAP and NAA were used in the medium and in the second part of experiment BAP and IAA were used. Comparative studies of the various hormonal concentrations were observed. The effect of 3 amino acids on regeneration was observed *viz.*, Glutamic acid, Tryptophan and Phenylalanine in combination with the optimal hormonal concentrations.

RESULTS AND DISCUSSION

The leaflet callus was tested with varying concentrations of BAP + NAA. It was noticed that when the concentration of BAP was increased from 0.5 mgL^{-1} to 6.5 mgL^{-1}, there was a marked change in the percentage of regeneration. Maximum percentage of regeneration was observed at 4.5 mgL^{-1} BAP + 0.5%mgL^{-1}NAA, while at lower and higher concentrations of 3.5 BAP with 0.5 mgL^{-1} NAA only green callus was observed. Raft development was observed at optimal concentrations of BAP + NAA. (Table 1). However, no significant regeneration response was seen at lower concentrations and green globular callus was observed at higher concentrations of BAP with 0.5 mgL^{-1} NAA.

When the same range of BAP concentrations were tested with 1.0 mgL^{-1} NAA increased regeneration response was observed, besides the type of morphogenesis was also found to be improved showing rhizogenesis along with raft formation. Thus the optimal concentration were found to be 4.5 mg L^{-1} BAP+1.0 mgL^{-1} NAA (Table 1). The requirement of higher NAA concentrations for rooting was also demonstrated by Gresshoff (1978), Bajaj (1981).

The response of leaflet explants was also tested with BAP + IAA, however the regeneration response of BAP + IAA was less as compared to that with BAP + NAA at similar concentrations (Table 2) with only friable green callus and globular structure development. The superiority of NAA over IAA in regeneration has been established by many workers in various plant species (Bajaj, 1990).

Three amino acids were tested for their response on regeneration, *Viz*; L-glutamic acid, Tryptophan and Phenylalanine. L-G.A was found to give the best regeneration response. The regeneration was maximum with BAP+NAA+L-G.A was prominent leaf buds on rafts with rooting (Table 3). Matsubara (1964) reported the stimulatory effect of L glutamic acid in regeneration from leaf cultures of *Datura petula*.

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Antifungal Properties of Some Plant-Extracts Against *Alternaria brassicae* (Berk.) SACC

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The aqueous extracts of twenty plants were screened for their antifungal activity against *Alternaria brassicae* (Berk). SACC., a causal organism of leaf spot diseases in Crucifereae and brown rot of cauliflower. The maximum inhibitory effect was shown by leaf extracts of *Curcuma domestica*. Some of the other plants showed intermediate inhibition against the mycelium growth of test fungi which varied in the following order *Clerodendron inerme*> *Acacia catechu*> *Anthocephalus cadamba*.

Keywords : *Alternaria brassicae*. Antifungal Activity, Plant-extracts, Phytochemicals.

INTRODUCTION

In order to maintain the productivity, more and more chemicals are being added in the natural environment, which enter the food chain through water, soil and air as a result it seriously affect the human health (Ramachandra and Nagarathna, 2003). As vegetables are the main dietary constituents in the Indian food, it becomes more important to search out alternatives to synthetic chemical control measures. Keeping these problems in view, efforts are underway to search economic safe phytochemicals, which could be utilized for disease control. Thus the object of present study was to exploit the potential of different plants extracts against *Alternaria brassicae* for selecting the most potent plant possessing antifungal activities.

MATERIAL AND METHODS

The various parts of each plant were collected from different places of Haryana and its neighboring states on the basis of their traditional values (Table-1). The collected plant materials were thoroughly washed with tap water, followed with distilled water and then kept in dark between the filter papers at room temperature till completely dry. Each plant sample was individually grounded into powder form for preparation of extract.

The fungi *Alternaria brassicae* used for the study was procured from the Division of Plant Pathology, IARI, New Delhi. The culture was maintained at 4°C on Yeast Glucose Agar medium with periodic sub-culturing.

Plant part extract (15% w/v) was prepared by brewing in hot water. 15 g dry powder of each plant sample was weighed and put in a cheese cloth bag and suspended in 100 ml of boiling distilled water for 20 minutes. The extract was allowed to stand for some time and decanted off into the flask and supernatant was used to assay the antifungal activity of each plant part extract by measuring the mycelium growth inhibition of test fungi as described by Bragulat *et al.,* 1991. A known volume of 15% plant sample extract was supplemented with yeast extract, glucose and agar. The medium was sterilized by autoclaving at 15lb. pressure for 15 minutes. Yeast Glucose Agar plates, without any plant extract supplementation,was run as a control. The test inoculum consisted of a disc 0.65cm. in diameter cut out from the edge of a growing fungal colony on glucose agar medium using a sterilized cork borer and placed at the centre of the agar medium in sterilized conditions. The experiments were conducted in triplicate along with equal number of control. The fungus was incubated at 26 ± 1°C and their growth diameters were measured after five days. The percentage inhibition was calculated by the formula as:

% inhibition = ((C–T) X 100/C)
where C = Diameter of control, T = Diameter of test

RESULTS

The activity of the plant-extracts against the mycelium growth of *Alternaria brassicae* is presented in Table 2. It is commonly observed that out of 20 plants parts extracts tested, rhizome extracts of *Curcuma domestica* has shown maximum inhibitory effect against the mycelium growth of *Alternaria brassicae* (60.0%). Three plants showed intermediate inhibitory effect against the mycelium growth of test fungus *i.e.,* leaf extracts of *Clerodendron inerme* (17.46%), root extracts of *Acacia catechu* (17.15%) and leaf extracts of *Anthocephalus cadamba* (11.43%). The other five plants have shown insignificant inhibition of mycelium growth against the test fungus and rest eleven plants samples did not show inhibitory activity.

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Table 1 : Common Names and Families of Plants used in Experiment.

Sr. No.	Botanical Name	Common Name	Name of Family	Distribution	Traditional Uses of Plants
1.	*Acacia arabica* Willd.	Kikar	*Mimosaceae*	India and Tropical Africa	Used for making furniture's, tanning, dyeing fabrics yellow, stem yields gum while seeds are fermented with dates to give beverages (Usher, 1971).
2.	*Acacia catechu* Willd.	Katha	*Minosaceae*	East India	Used in the treatment of diarrhea and throat infections (Usher, 1971).
3.	*Adhatoda vasica* Nees.	Adusa	*Acanthaceae*	Tropical India	A decoction of the leaves is expectorant and is used to relieve bronchitis (Usher, 1971)
4.	*Aegle marmelos* (L.)	Bael Patter	*Rutaceae*	India	A decoction of the leaves is a febrifuge and expectorant and is particulary used for asthmatic complaints. Also used to treat acute bronchitis, fever and dysentery (Dastur, 1962).
5.	*Albizia stipulata* Benthm.	Siris	*Mimosaceae*	Tropical Asia to Australia	The bark is used to treat boils and the leaves and seeds to treat diseases of the eyes (Usher, 1971).
6.	*Anthocepholus cadamba* (Mig.)	Kadam	*Rubiaceae*	Tropical Asia	The bark is used as a tonic and reduces fever (Usher, 1971).
7.	*Azadirachta indica* (A.)	Neem	*Meliaceae*	East India, Ceylon	Non-drying oil is extracted from the seeds. It is used for soap-making and to treat skin diseases, locally. The bark and leaf extracts are used as a tonic, and to reduce fevers (Usher, 1971).
8.	*Brassicae juncea* (L.)	Sarson	*Cruciferae*	Temperate Europe, Asia, introduced to N. America, Black Sea.	The oil (Ravinson Oil), extracted from the seeds. It is used locally as a luminant, Lubricant, and in the manufacture of soap (Usher, 1971).
9.	*Cannabis sativa* (L.)	Bhang	*Cannabidaceae*	Central Asia	Fibres used for cordage, sailcloth and caulking boat, seeds used in manufacture of paints, varnishes and soap, drug (bhang, hashish, ganja and marihuana) is produced. Its use is illegal in many countries (Usher, 1971).
10.	*Capparis decidue* (Roth.)	Karil	*Capparidaceae*	Sahara	Fruits eaten locally (Usher, 1971).
11.	*Carissa carandus* (L.)	Kraundha	*Apocynaceae*	India to Malaysia	The red, plum-like berries are eaten locally and made into jellies and preserves (Usher, 1971).
12.	*Cassia fistula* (L.)	Amaltash	*Leguminosae*	Tropical Africa	The pulp of pods is used as a laxative (Usher, 1971).
13.	*Casuarina equisetifoliu* (L.)	Chok/Jhau	*Casuarinaceae*	New South Wales, Quee--nsland, India Australia.	Wood is used for roof shingles and posting (Usher, 1971).

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14.	*Cedrela toona* (Roxb.)	Toon	*Meliaceae*	India to Australia	Flowers are source of a red and yellow dye, wood is used for furniture, house building, tea chests, oil casks and cigar box (Usher, 1971).
15.	*Ceiba pentandra* (Benth.)	Kapok Tree	*Bombraceae*	South America, India	The fibres are insect repellent: gum is laxative and used in bowel complaints, juice from its roots is a cure for diabeters (Vasishta, 1972).
16.	*Citrus limon* (Burmann.)	Nimbu	*Rutaceae*	Sub Tropical Asia, Greeks and Romanas, Azores, California and Italy	Fruits are good source of Vitamin C and B_1, carotene, Juice is extracted and used for fruit drinks, confectionery and flavouring, also a commercial source of citric acid. Lemon oil is used in perfumery, flavouring foods, flavouring liqueurs.
17.	*Clerodendron inerme* (Gaertn.)	Lanjai	*Verbenaceae*	Tropical and Sub-Tropical, India.	Used as blood purifier (Vasishta, 1972)
18.	*Colvillea wallichii* (L.)	Losara	*Boraginaceae*	India	Fruits are demulcient, expectorant and useful in bronchial affections and in irritation of urinary passages (Vasishta, 1972).
19.	*Curcuma domestica* (L.)	Haldi	*Zingiberaceae*	South Asia, India, China, East Indies and West Indies	Rhizome is a source of yellow dye. In India and Far East the juice is used for treating stomach complaints, bruises; fumes from the burning rhizome relieve cold and catarrh, and a paste of the rhizome accelerates the formation of scabs caused by smallpox and chickenpox (Usher, 1971).
20.	*Cuscuta reflexa* (L.)	Amar Bel	*Convolvulaceae*	Tropical and Temperate, India, Western Peninsula and Baluchistan.	Seeds are carminative and anthelmatic; plant used externally against itch, internally in protracted fevers; Infusion of the plant is used to wash sores (Vasishta, 1972).

DISCUSSION

Among the different plants whose extracts were found to be effective were rhizome extracts of *Curcuma domestica*, seed extracts of *Acacia catechu* and leaf extracts of *Clerodendron inerme*.

The rhizome extracts of *Curcuma domestica* found to be very effective against the test fungi. The plant is reported to possess various medicinal properties (Usher, 1971; Pandey, 1993) and various antifungal properties against phytopathogenic fungi (Ishratniaz *et al.*, 1994; Natrajan *et al.*, 2001); hence the extract of *Curcuma domestica* could be used for protecting plants against pathogenic organisms.

The antimicrobial activities of plants studied have also been reported by various workers in literature *i.e., Acacia catechu* (Singh and Sharma, 1978), *Aegle marmelos* (Ganesan *et al.*, 2004), *Albizia stipulata* (Dhar *et al.*, 1974) and *Cassia fistula* (Kavitha *et al.*, 2000).

Since the extracts of *Acacia arabica, Anthocephalus cadamba, Brassicae juanca* and *Clerodendron inerms* used in this study have not been tested before as inhibition of phytopathogenic fungi, therefore, they are the new addition to this study. The presence of various secondary metabolites such as alkaloids, quaternary alkaloids, coumarins, flavanoids,steroids/terpenoids, phenols *etc.* have been reported in the various plants extracts (Aswal *et al*, 1984; Abraham *et al.*, 1986; Chopra *et al.*, 1992) which may be responsible for the antifungal properties of the plants studied.

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Table 2 : Anti-fungal activity of Plant-extracts against *Alternaria brassicae* (Mean ± SD).

Sr. No.	Name of Plant	Part Used	Percentage Inhibition of Mycelium Growth
1.	*Acacia arabica* Willd.	Seed	6.25 ± 2.89
2.	*Acacia catechu* Willd.	Root	17.15 ± 2.97
3.	*Adhatoda vasica* Nees.	Leaf	-
4.	*Aegle marmelos* (L.)	Leaf	6.35 ± 3.25
5.	*Albizia stipulata* (Benthm.)	Seed	2.63 ± 4.11
6.	*Anthocephalus cadamba* (Mig).	Leaf	11.43 ± 2.39
7.	*Azadirachta indica* (A.) Juss.	Seed	-
8.	*Brassicae juancea* (L.)	Seed	6.78 ± 3.48
9.	*Cannabis sativa* (L.)	Leaf	-
10.	*Capparis decidua* (Roth.)	Seed	-
11.	*Carissa carandus* (L.)	Fruit	-
12.	*Cassia fistula* (L.)	Seed	7.14 ± 3.74
13.	*Casuarina equisetifolia* (L.)	Seed	-
14.	*Cedrela toona* (Roxb.)	Leaf	-
15.	*Ceiba pentandra* (Benth.)	Seed	-
16.	*Citrus limon* (Burmann.)	Seed	-
17.	*Clerodendron inerme* (Gaertn.)	Leaf	17.46 ± 2.44
18.	*Colvillea wallichii* (L.)	Leaf	-
19.	*Curcuma domestica* (L.)	Rhizome	60.00 ± 0.42
20.	*Cuscuta reflexa* (L.)	Whole Plant	-

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Relationship of *Solanum nigrum* Mosaic Virus a Strain of Cucumber Mosaic Virus with its Aphid Vector *Myzus persicae* Sulz

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Solanum mosaic virus a strain of cucumber mosaic virus (CMV) was detected in *S. nigrum* weed in and around cucumber crops in different farmers fields in Baghpat (U.P.) distt. Where cucumber mosaic virus has been a major problem in cucurbitaceous crops, they were tested for the presence of CMV by enzyme linked immunosorbent assay. Investigations carried out to determine the virus-vector relationship of *Solanum* mosaic virus indicated that, CMV was transmitted more efficiently by *M. persicae.* Adult of *M. persicae* in a group of 10/ test plants and given acquisition access for 10 minutes inoculation threshold of 60 minutes and pre-acquisition starvation 2 hours gave maximum infection. These studies indicated non-persistent relationship of CMV with its vector.

Keywords : *Solanum* mosaic virus a strain of CMV, Weed, Aphid vector, *Myzus persicae.*

INTRODUCTION

Volunteer plants and weed provides shelter and sources of nutrients for virus vectors. Numerous species of weeds that act as reservoirs for virus and its vectors have been found to be important in the epidemiology of certain diseases. (Johnson *et al.,* 1996; Fereres *et al.,* 1996, Lathan and Jones, 1991; Hobbs *et al.,* 2000). Many aphid species, especially *M. persicae* transmit CMV as a non-persistantly when the insect feed on an infected plant (Graham *et.al.,* 1979; Harris *et al.,* 2001). The aims of these studies was to determine the presence of the main viruses that infect cucumber crop on the *S. nigrum* weeds and to study the virus vector transmission of the pathogen from infected weed or cucumber by aphid vectors.

MATERIAL AND METHODS

The pure culture of *Solanum* mosaic virus was maintained on cucumber under insect proof glass house. The virus free colony of *M. persicae* was raised on cabbage sps. by single apterous aphid in the insect proof glass house. One-week-old cucumber plants were used in these experiments. Unless mentioned otherwise, both nymphs and adults were employed and reared glass house in this experiment. During the transmission feeding of aphids, test plants were covered with cylindrical iron cage wrapped with muslin cloth all round and top. The camel hairbrush was used for transmission of the aphid after given a stipulated period of acquisition feeding to the test plant. Aphids were killed after the required feeding period by spraying 0.05% dimethoate. The experiments were repeated twice.

RESULTS

1. Effects of pre acquisition fasting period of the aphid vector on the transmission *M. persicae* were collected from the cabbage plants and starved for 0, 5, 10, 15, 20, 40 and 60 minutes and 2 hrs. in a glass vials. There after the starved aphids were fed for 15 min. on virus infected plants of cucumber and 10 aphids from each group were released to healthy cucumber seedlings for inoculation feeding 15 min.
   The results indicated that the maximum transmission was obtained with 2 hrs. at pre-acquisition fasting period (Fig.1.).

2. Acquisition threshold of the aphid on the transmission efficiency of CMV.
   The results showed that the aphids acquired the virus within a short acquisition period of 30 sec. Higher percentage of transmission was observed with 10 min. and 15 min. Acquisition period and the transmission efficiency of aphid declined gradually with an increase in acquisition feeding period beyond 15 min. (Fig. 2).

3. Inoculation access period of the aphid vector.
   It is obvious that aphids were able to transmit the virus in as short as 30 sec. of inoculation feeding time. The efficiency of aphid increased with increase in inoculation feeding and higher percentage of transmission was observed when the aphids were given 15 min. of inoculation feeding period. (Fig. 3).

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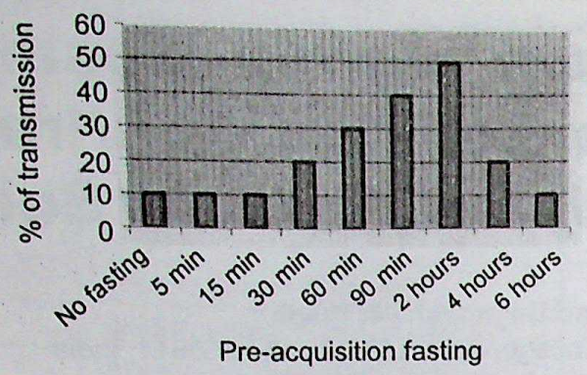

Fig 1. Effect of pre-acquisition fasting of aphid vector *M. persicae*

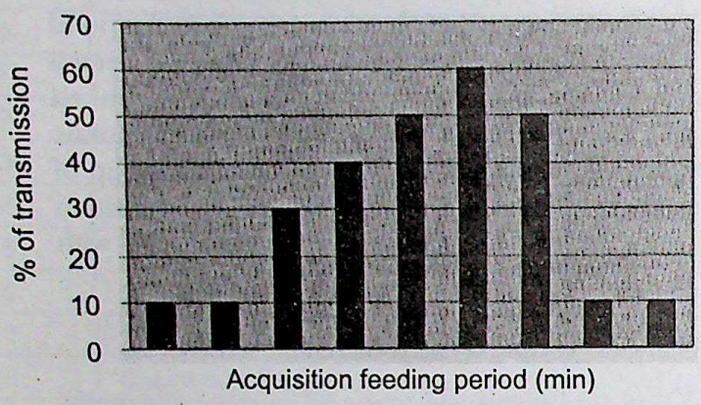

Fig 2. Acquisition threshold of the aphid on the transmission efficiency of aphid vector *M. persicae*

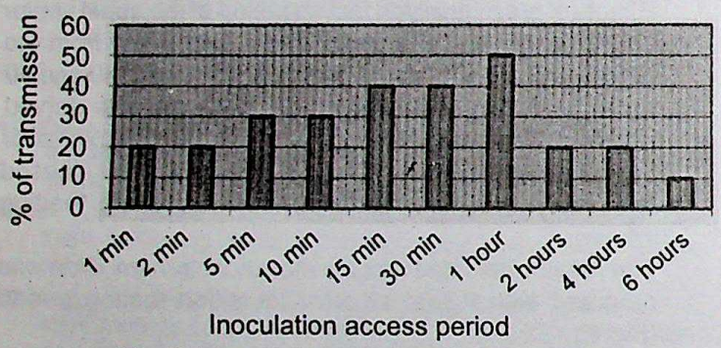

Fig 3. Inoculation access period of the aphid vector *Myzus persicae*

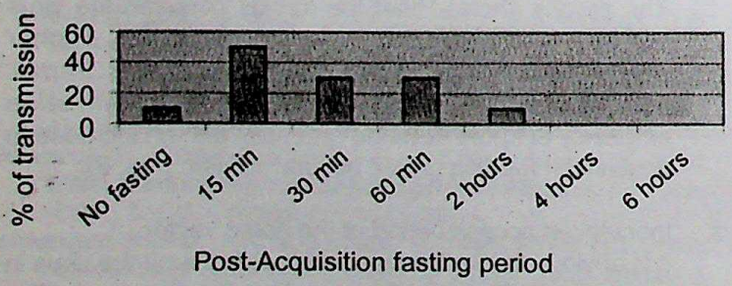

Fig 4. Effect of post-acquisition fasting of aphid vector *Myzus persicae*

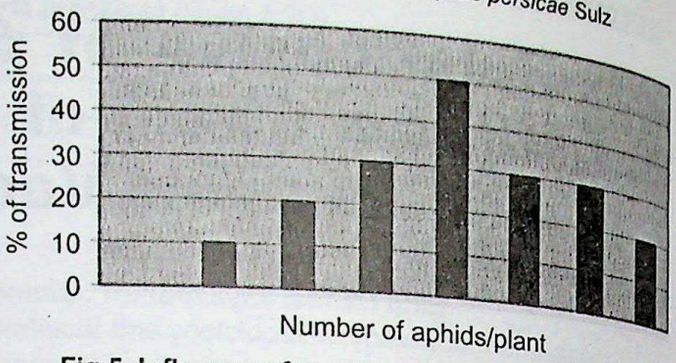

Fig 5. Influence of number of viruliferous aphids *Myzus persicae*

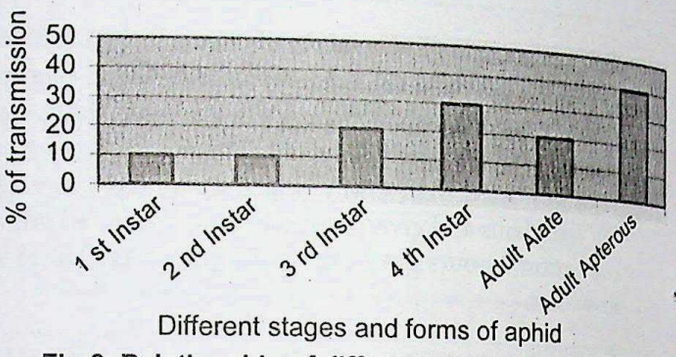

Fig 6. Relationship of different stages and forms of the aphid vector *Myzus persicae*

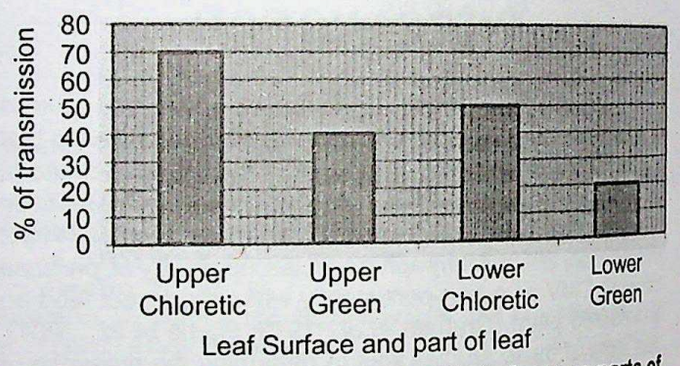

Fig 7. Comparison of chloretic with dark green parts of infected leaflets as virus source for aphid vector.

4. Effect of post acquisition fasting.
The results given in Fig. 4 showed that the maximum transmission was obtained when the vector was allowed inoculation access on test without post acquisition starvation and the aphids could remain viruliferous up to 4 hrs. and aphids became non-viruliferous beyond this period.

5. Influence of number of viruliferous aphids.
The data presented in Fig. 5 revealed that even a single aphid is enough to transmit the disease. The infection increased with in increase in number of inoculative aphids/ test plant. Maximum transmission was obtained with 10 aphids/plant. It was noticed that the transmission was reduced when longer number of aphids above the optimum level were released for inoculation.

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Table 1 : Influence of virus source.

Sr.No.	Host Plants	Number of Plants infected/Inoculated	Percentage of Transmission
1.	Cucumber	8 / 10	80.00
2.	*S. nigrum*	6 / 10	60.00

Table 2 : Comparision of chloretic with dark green parts of infected leaflets as virus source for aphid vector.

Sr.No.	Leaf Surface	Part of Leaf	Number of plants infected / inoculated	Percentage of Transmission
1.	Upper	Chloretic	7 / 10	70.00
		Green	4 / 10	40.00
2.	Lower	Chloretic	5 / 10	50.00
		Green	2 / 10	20.00

6. Relationship of different stages and forms of the aphid vectors.
The results given in Fig. 6 showed that all nymphal stages and both alate and apterous forms of aphid vector transmit the virus. The results also showed that the transmission was higher in the apterous adult forms than all nymphal stages and the alate adult forms of the aphid vector.

7. Influence of virus source.
To determine the information on the infection between the vector and virus source from host to test plant on cucumber. The result presented in the Table 1 indicated that cucumber proved to be the best host as virus source by the aphid vector.

8. Effect of leaf age and availability of CMV in chlorotic tissue.
The observation showed that the availability of CMV to aphids from the diseased leaves of Cucumber was found on 2nd leaf (70%) compared to 3rd (30%) and older leaves (20%) from the apex.
Also better transmission of virus from chlorotic part than green part of the same diseased leaf by the aphids was noticed (Table-2).

DISCUSSION

Investigation carried on the relationship for the CMV and its vector indicated that the increasing the pre acquisition fasting, acquisition and inoculation feeding period above the optimum decreases transmission percentage. There was an inverse relationship between the length of post acquisition fasting period and efficiency of the aphid vector to transmit the virus. Similar results were reported by Kassanis (1941), Sylvester (1955), Singh (1972), Dhingra and Chenulu (1980), Gupta and Chowfla (1986) and Tomer and Mohan (2001).

The present investigation have also indicated that maximum transmission was achieved when there is increase in the number of aphids and maximum percentage of infection was obtained when 10-15 aphids per plant were employed in the virus transmission. Similar results were obtained by different workers in case of different viruses. (Nariani and Sastary, 1962; Uperti and Nagaich, 1971; Singh, 1972; Ahlawat and Chenulu, 1982; Gupta and Chowfla, 1986; Tomer and Mohan, 2001).

The nymph and apterous adult were more efficient in transmitting the disease then alate adult. Similar result differences were obtained by Hamlyan (1953). Sylvester (1955), Singh (1972), Ahlawat and Chenulu (1982) and Tomer and Mohan (2001). They indicated that aphid vector *M. persicae* transmit the virus in a non-persistent manner. These finding are in agreement with those of Nagrajan and Ramakrishnan, (1971); Singh, (1972); Sharma and Raychoudhari, (1986); Ahlawat and Chenulu, (1982) and Tomer and Mohan (2001).

Availability of cucumber mosaic virus to aphids with the ageing of leaves and higher titer in chlorotic tissue compared to green tissue of the same leaves due to the propressive reduction in chlorotic areas. Similar results were obtained by Zettler (1969).

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Colonization and Spore Production by Mycorrhizal Fungi of Sesame

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Soil inoculation with VAM improved percentage of their colonization of sesame plants. External mycelium observed within the first few days was dimorphic with thin-walled and thick-walled hyphae. Chlamydospores were formed from only thick-walled hyphae. VAM spore number increased after 2 weeks and continued to increase up to 70 days. Internal mycelium was observed by 10th day and became extensively developed up to 25 to 30 days. Vesicles were formed from 2nd week. Arbuscules were seen after 5 - 6 days.

Keywords : Mycorrhizae, *Glomus, Acaulospora.*

INTRODUCTION

Mycorrhizae are symbiotic root-colonizing fungi benefiting the plants in absorbing phosphorus and contributing to the increased growth and nutrient uptake. They are important because most of the agricultural or forest soils are low in phosphorus content. Their hyphae extend beyond root apex into regions where roots cannot reach into the soils. They solubilize phosphate compounds for absorption and supply to root. Ectomycorrhizae form a mantle surrounding the roots while endomycorrhizae called vesicular - arbuscular endomycorrhizae (VAM) occur inside the roots producing vesicles and arbuscules to suck nourishment from host plant. Genera like *Glomus* form chlamydospores, zygospores and azygospores.

VAM endosymbionts are essentially not host specific, although some of them may form preferential association with certain host plants (Mosse, 1977). They give rise to extramatrical and intracellular hyphae which form (1) globose or terminal oval swellings called vesicles, (2) intracellular structures like small trees or arbuscules and (3) irregular clumps termed sporangia. They do not induce alterations recognizable with the naked eye except in roots of plants like onion and maize. The infection occurs solely in the epiblema and cortical parenchyma of roots which form a primary structure. Hyphae range from 2 - 27µm in diameter (Nicolson, 1959). They are either thin-or thick-walled, yellowish with typical unilateral angular projections and have non-septate vesicles like globose to obovate spores regularly associated with the extramatrical mycelium borne terminally or on short lateral branches.

The external vesicles vary from 20 - 50µm in diameter and are thick walled with a dense cytoplasmic content rich in oil globules. Hyphae can penetrate the epiblema of roots depending on the wall thickening of these outer cells of the roots. The outer cortical layer is often colonized by intracellular hyphae characterized by a linear or more often a looped arrangement without any signs of branching. Intracellular hyphae produce coils. Penetrating hyphal branches are usually found in the intermediate layers of the cortical parenchyma. These hyphae first spread in the intercellular spaces and are sometimes in bundles of 3 or 4. They run in parenchyma for considerable distances before penetrating cells giving rise to a complex hyphal branching system in the form of small bushes called arbuscules (Harley, 1969). The arbuscule has the most significant function as a site of fungus-root metabolic exchange (Cox *et al.,* 1975). Their presence is an indication of VAM infection in roots. At its senescence phase it disintegrates in the cell liberating nutrients into the cell. All endophytes also generally form vesicles which are intercalary or terminal swellings of hyphae of the VAM fungus. They are inter or intracellular found both in the inner and outer layers of cortex. They are mainly resting spores functioning like sporangia for reproduction. After senescence they get digested and their fats become utilizable in the host cells.

Vyas and Srivastava (1988) studied process of infection in moth bean by *Glomus fasciculatus.* VAM development in some grasses was studied by Ammani *et al.,* (1994). Mago and Mukerji 1994 have investigated the seasonal spore dynamics in Lamiaceae.

Colonization by VAM, their development and spore production in roots of sesame (*Sesamum indicum* L.) was studied in the present work.

MATERIAL AND METHODS

In the present study 5 VAM fungi namely *Glomus mosseae, G. epigaem, G. constricotum, G. monosporum* and *Acaulospora morrowea*, which were obtained from ICRISAT, Hyderabad, were employed for the artificial inoculation of two sesame cultivars namely Rajeshwari and Gowri.

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Dynamics of VAM infection and of the spore number in association with the growth of 2 cultivars of sesame was determined every 14 days starting with 2-weeks old seedlings extending up to 10 weeks-old plants (Table 1). The seedlings were raised in plastic pots, one set containing VAM - inoculated soil and another containing non-inoculated soil.

Estimation of infection percentage : Three plants of the same age from different corners of the fields were randomly selected. Their feeder roots were cut, washed and immediately preserved in FAA (1:1:18 = formalin : acetic acid : alcohol). Clearing and staining of roots was done following the procedure of Phillips and Hayman (1970) with some modifications, (1) In one set, the roots were cut into pieces of 7 cm length. They were kept in autoclavable vials containing 10% KOH and autoclaved at 121°C for 10 minutes. After autoclaving, KOH was decanted out and 1% HCl was added and allowed to stand for 5 to 10 minutes and again decanted. In another set, the roots were rinsed in water, then kept for 5 minutes in 5% lactic acid and decanted. Bleaching of the heavily pigmented roots was carried out as described by Rajapakse and Miller (1992). The cleared roots were stained by adding Tryphan blue (0.08% tryphan blue in lactophenol) and allowed to stand for 15-20 minutes. Later the stain was decanted and stained roots were collected and arranged in a Petri dish having gridlines (Newmann, 1966). The spread out roots were immersed in glycerol: water (1:8) to prevent dessication and also to increase the viscosity of the system. The stained roots were observed under a microscope for vesicles and arbuscules of VAM. The Petri plate was first moved horizontally and then vertically along gridlines. Total number of the roots and infected roots intersecting gridlines were recorded. Percentage of infection was calculated by the formula given by Giovannetti and Mosse (1980).

$$\text{Percentage of mycorrhizae infection} = \frac{\text{Total number of infected roots intersecting gridlines}}{\text{Total number of roots intersecting gridlines}} \times 100$$

Formation of VAM resting spores: Resting spores of VAM fungi consisting of sporocarps, chlamydospores, azygospores and soil-borne vesicles were extracted by wet sieving and decanting as described by Gerdemann and Nicolson (1963) and Pacioni (1992). For quantitative and qualitative estimation of VAM resting spores, 100 g of rhizosphere soil including the roots was collected from sesame plants. The collected soil was put into 500 ml of water and shaken gently allowing it to settle for few seconds. The supernatant liquid was poured through a coarse soil sieve (500-800 mm) to remove large pieces of organic matter. The lower collected liquid was passed through different sieves (250, 106 and 45 micropores). This process was continued till all colloidal materials passed through the sieves. All the debris collected on sieves was taken into a petridish and observed for resting spores under the stereobinocular microscope. Spores were carefully extracted with needles and brushes and their slides were made in lactophenol for identification. The resting spores were identified with the help of key provided by Hall (1980) and Schenck and Perez (1988).

RESULTS AND DISCUSSION

Spore number of VAM in the rhizosphere and percentage of colonization was more in inoculated plants than in non-inoculated soils. It steadily increased up to 70 days, which is flowering time of host plant. Root started decaying after flowering and as the cortex is sloughed off, vesicles were released into the soil as spores. Similar results were reported by Mago and Mukerji (1994) who investigated the seasonal spore dynamics of VAM in Lamiaceae. However, Vyas and Srivastava (1988), who studied process of infection by *Glomus fasciculatus* found in moth bean that formation of external and internal mycelium, infection pegs, arbuscule and vesicles formations were quite early.

Infection occurred in sesame through the epiblema cells by the formation of infection pegs and appressoria. Internal mycelium was observed by 10th day and became extensively developed in 25 to 30 days. Sutton (1973) noted that an initial lag phase of 20 to 25 days was needed for spore germination, germ-tube growth and penetration of host root. A lag period of 30 days after inoculation of spores of *Glomus fasciculatum* was attributed to spore germination and initiation of infection. Ammani *et al.*, (1994) observed that VAM fungi started colonizing roots from 20 days after sowing in *Choris borbala* and from 30 days in *Echinochloa crushgalli*. A time lag of one day between hyphal contact and subsequent penetration was reported by Brundrett *et al.*, (1985).

External mycelium was observed within the first few days, but develops little late *i.e.*, 5 - 6 weeks after infection of sesame roots. The external mycelium possessed dimorphic thick-walled and thin-walled hyphae. Ammani *et al.* (1990, 1994) who studied VAM in grass observed dimorphic mycelium after 50 days. Soil-borne chlamydospores were formed from only thick-walled / hyphae. External chlamydospores were found to be formed in cotton roots after 90 days (Nirmala and Mohan Kumar, 1990).

Thick-walled ovate vesicles were formed from 2nd week of infection of sesame plant roots and continued till the end of investigation.

Arbuscules formation started from hyphae in 5 - 6 weeks old sesame plants. But later these arbuscules disappeared within 3 - 5 days. Ammani *et al.*, (1990) reported in pearl millet that arbuscules appeared only after 40 days and increased gradually with a slight decrease after 80 days. Similarly Nirmala and Mohan Kumar (1990) found in cotton that arbuscular infection was abundant in the roots of 60 days-old plants.

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Table 1 : Dynamics of VAM spore number in the rhizosphere and percentage of colonization of two artificially inoculated sesame cultivars (Rajeshwari and Gowri).

Age of sesame plant (in weeks)	Spore number / 100 g soil				Percentage of colonization			
	VAM inoculated soil		VAM Non-inoculated soil		VAM inoculated soil		VAM Non-inoculated soil	
	Rajeshwari	Gowri	Rajeshwari	Gowri	Rajeshwari	Gowri	Rajeshwari	Gowri
2	50	40	15	15	45	20	20	0
4	83	55	25	38	52	43	35	20
6	155	80	45	50	58	55	38	35
8	198	156	152	95	65	58	48	38
10	252	195	180	130	60	60	55	42

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Influence of AM Fungi on Growth Parameters and Chlorophyll Content of Wheat (*Triticum aestivum* L.)

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Mycorrhiza is the mutualistic symbiosis between soil borne fungi and roots of plants. The most important and widely distributed type of mycorrhiza is arbuscular mycorrhiza (AM). The present study was undertaken to find out the effect of the AM fungi on the growth and biochemical constituents in wheat. Due to AMF association, the survivability of the wheat was very high as compared to wheat grown under control condition. In this study it was observed that plants inoculated with AMF have grown taller compared with non-inoculated plants. The growth parameters such as shoot length, root length, vigour index, dry weight of roots, dry weight of shoot, stem girth and number of leaves per plant were found increased due to the influence of AM fungi as compared to control plants. There was significant increase in per cent root colonization and total chlorophyll content in AM inoculated plants than control plants. The present investigation conforms that inoculation of AMF was found to be more promising to induce the growth of wheat plant.

Keywords : AMF, Chlorophyll, Dry weight of root, Dry weight of shoot, *Glomus mosseae.* Root length, Shoot length, *Triticum aestivum*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second most important cereal just after rice. It is eaten in various forms by more than one thousand million human beings in the world. In India it is second important staple food crop, which contain a high percentage of carbohydrates and proteins.

The arbuscular mycorrihizal fungal (AMF) association is known to improve plant growth through better uptake of nutrients, tolerance to drought and salinitly (Harley and Smith, 1997). The mycorrhizal mutualistic symbiosis between plant roots and soil fungi is known to play a significant role in uptake of P by plants (Barrow and Rocadri, 1977). Among mycorrhizae, AM fungi, a fungal biofertilizer is the most promising and extensively used to enhance the growth in different crop plants (Marwaha, 1995; Inchal and Lakshman, 2006). AMF biofertilizer is a natural product carrying living micro-organisms derived from the plant root or cultivated soil. As such, no harmful effect on soil fertility or plant growth is generally discernible (Sen, 2005). The beneficial effects of AMF have been reported in many leguminous plants and other crop plants (Mamatha and Bagyaraj, 2001). AMF help in plant nutrition, disease resistance and also provide an alternative to chemical fertilizers particularly in land reclamation, it habitat restoration and sustainable agriculture. The AM association is seen in most grasses, cereals and millets (Ammani, 1989). There is more work on the incidence of AMF in relation to crop plants (Bagyaraj and Varma, 1995), cereal crops (Ammani *et al.* 1985), Vegetable plants (Creighton *et al.*, 1986) and cash crops. Most horticultural plants are colonized by arbuscular mycorrhizal fungi (AMF) whose presence can enhance the growth of the host plant (Ortas and Verma, 2008). The increase in stomatal behavior and photosynthesis of host plants along with increase in the chlorophyll concentration due to inoculation of the AM fungi in different plant species is well documented. (Allen *et al.*, 1981; Panwar, 1991).

The present study was undertaken to find out effect of the AM Fungal species *Glomus mosseae* (GM) on the growth performance of wheat (*Triticum aestivum* L.) plant.

MATERIAL AND METHODS

Plant material and AM inoculum : A pot experiment was carried out in the Department of Botany, New Arts, Commerce and Science College, Ahmednagar (Maharashtra) during the year 2009/10 to study the effect of AM inoculation (GM) on *Triticum aestivum*. Soil based culture of AMF, (*Glomus mosseae*) were multiplied in Jowar roots. The seeds of *Triticum aestivum* L. var. 496 used as a experimental plant. Healthy seeds of wheat were taken, surface sterilized with 0.1% $HgCl_2$ washed with distilled water thrice and seeds were soaked in

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the distilled water for 4 hours. Fifteen seeds were sown in each pot.

Experimental design : Pots of 30 x 30 cm size were filled with 15Kg of sterilized sandy loam soil. Pots were arranged in completely randomized block design. The experiment was replicated thrice. For AMF (*Glomus mosseae*) four treatment as 25 g, 50 g, 75 g and 100 g of mixed inoculum was placed just below the 5 cm in experimental pots (containing 250-300 spores/100 g inoculum). Control was maintained without application of AMF. The plants were irrigated once in two days. Observations were recorded at seedling and anthesis stage after AMF inoculation. Shoot length, root length, number of leaves, dry weight of root, dry weight of shoot, girth of stem and the percentage of root colonization as described by Phillips and Hayman (1970). Total chlorophyll was determined by Arnon's (1949) method and amount of chlorophyll was expressed in mg/g fresh weight. The vigour index was determined by multiplying the germination percentage with the sum of root and shoot length (Woodstock, 1969).

RESULTS AND DISCUSSION

In this study, it was observed that plants inoculated with AMF (GM) have grown taller than the non-inoculated plants. The growth parameters such as root length, shoot length and stem girth were found increased due to the influence of AMF (GM) (Table 1). The significant increased root length (10.70cm) and shoot length (22.44cm) were observed in 75g AMF inoculated plants as compared to control. Similar observations were made by Chiramel *et al.,* (2006), as plants inoculated with *Glomus leptotrichum, G. etunicatum* and *G. mosseae* showed higher plant height, root length and shoot length as compared to non-inoculated control plants. Stem girth was also significantly more (1.46cm) in plants inoculated with 75g *Glomus mosseae* compared to control plants. Role of VAM fungi in improving the stem girth and plant biomass is well documented in tropical plantation crops like citrus and mango (Manjunath *et al.,* 2001).

The growth character such as number of leaves per plant was found increased due to the influence of AMF (GM) fungi. It is clear from the results (Table 1), that all the GM inoculated plants showed higher leaf number as compared to control plants at seedling and anthesis stage. The results were supported by the work of Boby and Bagyaraj (2003), who have found the enhanced growth of *Coleus farskohlii* with the dual inoculation of AM fungi and PGPR organisms. The results (Table 1) revealed that all the GM inoculated plants showed more root and shoot dry weights as compared to control plants. An increase in dry weight of root and shoot (4.70 and 10.32g respectively) was observed in 75g GM inoculated plants as compared to control plants during anthesis stage. The treatment of GF recorded good shoot and dry weights and the similar results were shown in soybean and cowepea when inoculated with AMF (Gupta *et al.,* 1999).

The data on percentage increase of mycorrhizal colonization due to inoculation of AMF (GM) are given in Table1. The percentage root colonization due to AMF (100g) was higher (73.00%) as compared to control plants (30.33%) during anthesis stage. This root colonization by AMF showed significantly improved phosphorous uptake per unit root length due to the enhancement of the total root surface by hyphal growth (Li, *et al.,* 1991).Significantly higher amounts of the total chlorophyll (1.246 and 5.183 mg/g fresh wt.) were observed in AMF (75g) inoculated wheat plant during seeding and anthesis stage as compared to non-inoculated control as shown in Table 1. Similar result was observed by Allen *et al* (1981) in *Bouteloua gracilis*. The data on vigour index due to inoculation of GM are given in Table 1. It is clear that all the treated plants have shown better vigour index over control plants at seedling and anthesis stage.

Hence, it can be concluded from the present investigation that inoculation of *Glomus mosseae* was found to be more promising to induce the growth of the wheat plant. The study also shows that AM fungi help wheat perform better. The strategy of using AM fungal species as a biofertilizer will help improve the growth and yield of wheat plant.

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Table 1 : Effect of AMF (GM) on morphological parameters, AM root colonization, total chlorophyll content and vigour index of Triticum aestivum L.

Treatment	Root length (cm)		Shoot length (cm)		Stem girth (cm)		No. of leaves (per plant)		Dry wt. of roots (g)		Dry wt. of shoots (g)		AM colonization in roots (%)		Total chlorophyll content mg/gm fresh wt.		Vigour Index	
	Seedling Stage	Anthesis Stage	Seedling Stage	Anthesis Stage	Seedling Stage	Anthesis Stage	Seedling Stage	Anthesis Stage	Seedling Stage	Anthesis Stage	Seedling Stage	Anthesis Stage	Seedling Stage	Anthesis Stage	Seedling Stage	Anthesis Stage	Seedling Stage	Anthesis Stage
C	2.11	4.2	7.12	10.53	0.22	0.52	4	6	0.26	0.92	1.78	4.92	0	30.33	0.67	2.86	830.07	1325.07
25	3.53*	5.24*	8.36*	11.7*	0.78	1.23	4*	7*	0.64*	1.50*	1.98*	5.60*	10	45.66*	0.78*	3.32*	1189	1694*
50	4.5	6.23*	9.21	14.5*	0.8	1.32	5*	7*	1.12*	2.34*	3.02*	7.64*	12	55	0.84*	3.64*	1371	2073*
75	5.1	10.7	10.34	22.44	0.96	1.46	6	8	1.76	4.7	4.76	10.32	14.33	70.66	1.25	5.18	1544	3314
100	4.68	10.3	9.5	21.62	1.02	1.45	5*	8	1.9	4.62	4.98	9.8	19.66	73	0.85*	3.65*	1418	3192
SE ±	0.53	1.33	0.55	2.49	0.14	0.17	0.37	0.37	0.32	0.79	0.67	1.08	3.23	7.95	0.1	0.39	123.96	399.46
CD at (0.05)	1.49	3.71	1.52	6.91	0.39	0.48	1.04	1.04	0.88	2.19	1.87	3.01	8.98	22.11	0.27	1.09	344.6	1110.5
Total	3.60	7.91	8.64	17.44	0.61	1.00	5.04	7.04	1.14	3.11	3.65	7.93	8.98	52.44	0.94	3.94	1174.57	2435.57

*Indicates that these values are Statistically Non Significant over the Control.
Remaining all the values are Statistically Significant over the Control.
Values are mean of triplicates
AMF-Arbuscular Mycorrhizal Fungi; G.M. *Glomus Mosseae*, C-Control.

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Role of Storage Mycoflora on Deterioration / Loss of Cellulose of *Diospyros melanoxylon* Roxb. (Tendu) Leaves

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Present study deals with leaf surface mycoflora of *Diospyros melanoxylon* Roxb. (Tendu) in stored condition. Stored leaves were collected from different areas of Durg, Rajnandgaon and Bastar forest divisions. Leaves were found infested with various molds dominated over by *Aspergillus niger, A. flavus. Curvularia lunata, Pestaloria machrotricha, Mammaria echinobotryoides* and *Chaetomium globosum.* Out of these six (06) isolates, *Chaetomium globosum* was capable to deteriorate cellulose up to 80% *Mammaria echinobotryoides* and *Curvulaia lunata* were capable to deteriorate cellulose up to 58%. *Pestalotia machrotricha* was capable to deteriorate cellulose up to 51%, while *Aspergillus niger* and *Aspergillus flavus* were found to deteriorate cellulose up to 44% - 48%. Hence *Chaetomium globosum, Mammaria echinobotryoides* and *Curvularia lunata* were found to play important role in deterioration/loss of cellulose during storage of tendu leaves.

Keywords : Cellulose, deterioration/loss, Storage mycoflora, Forest divisions, Fungal isolates.

INTRODUCTION

Diospyros melanoxylon Roxb. (Tendu) is one of the most important forest produce of Chhattisgarh. Dried leaves of tendu are mainly used in preparation of "bidi" provides a major financial support to rural and tribal inhabitants. The leaf surface is an important surface for the growth of various fungal organisms, as it easily provides essential nutrients required for their life and growth. The nutrients of fungal organisms are available on the surface of living as well as dead leaves in form of organic and inorganic substances.

Fungi simply degrade the organic substances to yield compounds that are readily available to fulfill their own nutritional and metabolic requirements. The pathogenic leaf surface mycoflora utilize and exploit nutrients according to their utilization efficiencies there by lowering quality as well as useful part of the leaves.

Thus, present investigation deals with the quantitative estimation of deterioration/loss of cellulose mainly caused by storage mycoflora of *Diospyros melanoxylon* Roxb. (Tendu) leaves.

MATERIAL AND METHODS

Fungi were identified according to the mycological manuals (Dematiaceous Hyphomycetes - Ellis, 1971 and 1976; Introductory mycology - Alexopoulos, 1978; Illustrated genera of imperfect fungi - Barnett and Hunter, 1987; A manual of soil fungi - Gilmann, 1995 etc.)

Quantitative estimation of deterioration/loss of cellulose was done following Goering and Van Soest (1970) scheme of analysis of forage.

RESULTS AND DISCUSSION

Quantitative estimation of deterioration/loss of cellulose in healthy and infested leaves showed the following results. Healthy leaves showed 38.19% DM cellulose, while infested leaves showed decrease in cellulose contents.

The decrease in cellulose content for infested leaves of tendu was 7.98% DM (20.91% loss), 8.20% DM (21.48% loss), 10.49% DM (27.48% loss), 07.09% DM (18.58% loss), 12.49% DM (32.72% loss) and 16.74% DM (43.86% loss) by *Aspergillus niger, A. flavus, Curvularia lunata, Pestalotia machrotricha, Mammaria echinobotryoides* and *Chaetomium globosum* respectively after five days of incubation.

Similarly decrease in cellulose in infested tendu leaves was 11.31% DM (29.63% loss), 12.99% DM (34.03% loss), 16.86% DM (44.17% loss), 13.72% DM (35.95% loss), 16.81% DM (44.04% loss), and 23.77% DM (62.28% loss) after ten days of incubation and 16.80% DM (44.01 % loss), 18.40% DM (48.21% loss), 21.96% DM (57.54% loss), 19.63% DM (51.43% loss), 22.09% DM (57.88% loss) and 30.90% DM (80.96% loss) after fifteen days of incubation, when infested with *Aspergillus niger, A.flavus, Curvularia lunata, Pestalotia machrotricha, Mammaria echinobotryoides* and *Chaetomium globosum* respectively.

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The anova table shows that the variation in the percentage loss of cellulose due to incubation period is significant at 5% level. The table also shows that the variation in the percentage loss of cellulose due to species is significant at 5 % level.

In the present study all the test organisms were found to have potentiality to degrade cellulose quite efficiently. Quantitative estimation of cellulose contents of the tendu leaves after infestation showed that the infested leaves showed a tendency to decrease its contents. Results indicate that *Chaetomium globosum, Mammaria echinobotryoides, Curvularia lunata* and *Pestalotia machrotricha* were capable to deteriorate cellulose up to 50-80%, while *Aspergillus niger* and *A. flavus* were capable to deteriorate cellulose up to 40-50%.

The results indicated that *Chaetomium globosum, Mammaria echinobotryoides* and *Curvularia lunata* could be more harmful for cell components of the tendu leaves. Decrease in cell wall components of infested stroed substrate is quite common observation. The concep of cell wall deteriorating enzymes being involved in the host parasite interaction of higher plants and microbial pathogen has also been confirmed and elaborated by Dimond (1970), Wood (1970) and Mullen & Bateman (1975).

The cellulose fibrils by intertwining make a porous lattice like structure, which forms the structural framework of the cell wall. The submicroscopic spaces between the fibrils are filled with pectic materials, lignin and hemicellulose in varying amount (Albersheim, 1965). Several enzymes secreted by a number of pathogen *in vitro* and *in vivo* are known to deteriorate cell wall (Wood, 1970). The growth of fungi on ligno-cellulosic materials depends on the production of many enzymes, one of the most important being the cellulase system (Malan, 2004; Aro *et. al.,* 2005; Schwarze, 2007). Siu and Sinder (1951) have reported that *Myrothecium* species showed high rate of cellulose decomposition. Levi and Preston (1965) have reported that the fungus *Chaetomium globosum* causes extensive loss in weight, removing primary polysaccharides on beech wood. Mandels and Reese (1964) have been found that with cellulosic materials, enzymes released by fungi and / or bacteria essentially cause biodeterioration.

Meyers and Scott (1968) reported 54% weight loss of cellulose within six days for *Dendryphiella salina* (P-16) when grown on T.V.M. medium, this figure rose up to 88% weight loss by 21 days. Chang (1967) reported that *Chaetomium thermophile* and *Aspergillus fumigatus* caused a high loss in weight of filter paper when incubated at 45ºC for 3 weeks. Fergus (1969) also reported that *Chaetomium thermophile* var. *coprophile* and *Chaetomium thermophile* var. *dissitum* caused high loss in weight of filter paper when incubated for 25 days at 45ºC.

Meyers (1968) gravimetric data were presented showing significant weight losses (50% within three weeks) of cellulosic substrates with growth of representative *Ascomycetes* and *Deuteromycetes.* Garrett (1974) has studied the cellulose decomposing ability of foot rot fungi caused by *Rhizoctonia solani*. Olutiola (1976) have studied cellulose complex of many fungi including *Aspergillus sydowi, A.tamarii* and *Penicillium citrimum* extensively. Person *et al.,* (1991) observed the species of *Trichoderma, Aspergillus, Sporotrichum, Fusarium* and *Penicillium* are wall known producers of cellulase.

Decomposition of teak leaf by some fungi studied by Vittal Rao (1997) has found that cellulose content of teak leaf decreased due to fungal infestation and concluded that the fungus, *Chaetomium globosum* was responsible for maximum decrease in the cellulose content of the leaf. Gayal and Khandeparkar (1998) studied the production of cellulase by *Penicillium funiculosum*.

The result of the present study reveals that all the six test fungal organisms were found to able to deteriorate cellulose (Chief cell wall component) of tendu leaves during storage. The percentage loss of cellulose of the tendu leaves increase with storage period. Out of six test fungal organisms, three *i.e., Chaetomium globosum, Mammaria echinobotryoides* and *Curvularia lunata* were found to cause more deterioration/loss in cellulosic contents.

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Table 1 : Quantitative Estimation of Total Cellulose in Healthy and Infested Leaves with Percentage Loss due to Infestation.

Category of Sample	Incubation Period								
	5-Days incubation			10-Days incubation			15-Days incubation		
Leaves infested with	Cellulose content (% DM basis)	Loos of Cellulose (% DM basis)	% Loss	Cellulose content (% DM basis)	Loss of Cellulose (% DM basis)	% Loss	Cellulose content (% DM basis)	Loos of Cellulose (% DM basis)	% Loss
1. *Aspergillus niger* Van Tieghem.	30.21 ± 0.02	07.98 ± 0.12	20.91%	26.89 ± 0.13	11.31 ± 0.25	29.63%	21.39 ± 0.09	16.80 ± 0.20	44.01%
2. *Aspergillus flavous* Link.	29.99 ± 0.13	08.20 ± 0.24	21.48%	25.20 ± 0.12	12.99 ± 0.19	34.03%	19.80 ± 0.13	18.40 ± 0.24	48.21%
3. *Curvularia lunata* Boedijn	27.71 ± 0.06	10.49 ± 0.17	27.48%	21.91 ± 0.10	16.86 ± 0.06	44.17%	16.24 ± 0.06	21.96 ± 0.08	57.54%
4. *Pestalotia machrotricha* De Not.	31.11 ± 0.15	07.09 ± 0.21	18.58%	24.96 ± 0.13	13.72 ± 0.25	35.95%	18.56 ± 0.12	19.63 ± 0.23	51.43%
5. *Mammaria echinobotryoides* Cesati.	25.70 ± 0.02	12.49 ± 0.13	32.72%	21.38 ± 0.03	16.81 ± 0.11	44.04%	16.11 ± 0.10	22.09 ± 0.21	57.88%
6. *Chaetomium globosum* Kunze.	21.45 ± 0.12	16.74 ± 0.24	43.86%	14.43 ± 0.03	23.77 ± 0.14	62.28%	07.30 ± 0.07	30.90 ± 0.18	80.96%
HEALTHY LEAF	39.19 ± 0.12								

Data presented is mean of 3 replicates

Analysis of Variance for two way ANOVA

Source of Variation	Sum of squares (SS)	Degree of freedom (df)	Mean squares (MS)	F-ratio	5% F - limit
Between incubation period	2569.51	2	1284.75	293.32	4.10*
Between species	1922.02	5	384.40	87.76	3.33*
Error	43.82	10	4.38		
Total	4535.35	17			

* - Significant

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Response of Arbuscular Mycorrhizal (AM) Fungi on Growth of Cultivated Medicinal Plants

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A study has been undertaken to evaluate the response of five indigenous AM inoculants on growth of Sarpgandha (*Rauwolfia serpentina*), Vasaka (*Adathoda vasica*), Pathrchur (*Coleus aromaticus*) and Kalmegh (*Andrographis peniculata*). Seedlings of each plant species were raised in polythene bags, transplanted in sterilized soil in earthen pots, and inoculated with 10 g of soil containing 25 - 30 spores of pure inoculum. All the plants in general responded well towards AM inoculation over control. However, the extent of growth varied with the species of AM fungi and host plant. *G. mosseae* recorded its superiority with *Coleus aromaticus*, *Adathoda vasica* and *Andrographis peniculata*. Inoculation of *A. appendiculata* was found superior for *Rauwolfia serperntina*. The improvement in plant growth could be attributed to the enhancement of the plant to absorb more nutrients via an increase in the absorbing surface area.

Keywords : AM Fungi, Growth, Medicinal plants, Biofertilizer.

INTRODUCTION

Research on soil microorganisms as biofertilizers, for increase in biomass. has received many attention of plant growers. Mycorrhizal fungi are known to improve the nutritional status, growth and development of plants, increase phosphorus uptake, protect plants against root pathogens and offer resistance to drought and salinity (Gupta *et al*, 1997; Vijaya, and Srivasuki, 1997; Nadagouda *et al.*, 2006; Chandra *et al.*, 2007; Kumudha and Gomathinayagam, 2007; Singh *et al.*, 2007; Arshi and Roy, 2008; Meena *et al.*, 2008). Although these symbionts are known to be cosmopolitan and non-host specific, several reports are available indicating their preference towards different hosts and environment. Utilization of biofertilizer is a promising alternative to chemical fertilizers. Now-a-days medicinal plants are in great demand due to increased acceptance of Ayurveda and traditional medicines because of less or no side effects. In order to maintain sustanied supply of raw materials to the drug industry, the medicinal plants are now cultivated in large scale. The current day emphasis is on sustainable agriculture, which uses less of chemical input like fertilizers and pesticides having adverse effects on soil health and environment. Host preference towards mycorrhization has been reported in a few medicinal plant species like *Coleus forskohlii* (Gracy and Bagyaraj, 2005), *Andrographis paniculata* (Chiramel, 2006), *Catharanthus roseus* (Mishra *et al.*, 2006) and *Stevia rebadiana* (Sharma *et al.*, 2007). *Spilanthes acmella* (Kumar *et al.*, 2008).

Durg is situated in the central plains of Chhattisgarh having tropical climate. The cultivated lands are mainly used for paddy but many farmers have shifted to vegetable farming as well as cultivation of medicinal plants for better economic returns. State Vanoshadhi Board has shortlisted some key medicinal plants suitable for Chhattisgarh.

In the present investigation, the response of indigenous AM fungi on growth of Sarpgandha (*Rauwolfia serpentina*), Vasaka (*Adathoda vasica*), Pathrchur (*Coleus aromaticus*) and Kalmegh (*Andrographis peniculata*) has been evaluated.

MATERIALS AND METHODS

The AM fungi were isolated from rhizospheric soil of different medicinal plants. Mass propagation of pure AM fungal spores was done initially using onion bulbs in polythene bags and then wheat as a host plant in earthen pots. After six weeks of growth, soil containing hyphae, spores, and root bits were dried and used as inoculum. Seedlings of medicinal plant species were raised in sterilized sand: soil mixture (1:3) in plastic trays. Seed of *Adathode vasica*, *Rauwolfia serpentina* and *Andrographis peniculata* were surface sterilized by mercuric chloride (0.01%) and cuttings of *Coleus aromaticus* were treated with Twin 20 (0.05%). The seedlings were provided with sterilized water and Hoagland solution periodically to ensure proper growth. Seedlings of equal size were transplanted in sterilized soil in earthen pots and inoculated with 10 g of soil contaning 25 - 30 spores of pure inoculum. Observations were recorded for spore population/ 100 g soil (Gerdeman and Nicolson 1963), % Root colonization (Phillips and Hayman 1970; Kormanic and Mc Graw, 1982), Dry Biomass (g) and Root length (cm) of plants after 45 and 90 days of inoculation. Ten replicates were maintained for each experiment.

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RESULTS AND DISCUSSION

The effect of different AM inoculants on the growth of medicinal plants after 45 and 90 days of inoculation is depicted in Tables 1 - 4. All the plants showed positive response in terms of percentage root colonization, spore number, root length and total dry biomass towards inoculation over control.

1. *Rauwolfia serpentina* - A significant increase was observed in percentage root colonization and AM spore number after 90 days of inoculation. Maximum percentage root colonization was observed in *G. mosseae* inoculation (78 ± 1.39) whereas *G. aggregatum* and *A. appendiculata* responded best (345 ± 1.48 and 345 ± 1.18 respectively) in terms of spore number per 100g of soil. Maximum root length and total dry biormass was found supported by *A. appendiculata* (75.4 ± 1.82 and 55.7 ± 1.27 respectively) followed by *G. aggregatum* (74.2 *G. masseae* 1.12) for root length and *G.mosseae* (49.7 ± 1.46) for dry biomass.

2. *Adathoda vasica* - *G. mosseae* supported the best of all in terms of percentage root colonization (78 ± 1.22), AM spore number (320 ± 1.31), root length (40.5 ± 1.145) and total dry biomass (32.8 ± 1.21) after 90 days of inoculation. *A. appendiculata* secured second position to support percentage root colonization (70 ± 1.14) and AM spore number (297 ± 1.26), *G. macrocarpum* and *G. aggregatum* for root length (34.6 ± 0.93) and total dry biomass (28.5 ± 1.11) respectively.

3. *Coleus aromaticus* - *G. mosseae* was recorded the best inoculant over control in terms of percentage root colonization (58 ± 1.35), AM spore number (245 ± 1.92), root length (27.56 ± 1.01) and total dry biomass (35.93 ± 1.04) after 90 days of inoculation. This was followed by response of *A. appendiculata* for percentage root colonization (56 ± 0.21), AM spore number (231 ± 1.05), root length (25.71 ± 1.20) and total dry biomass (28.51 ± 1.13).

4. *Andrographis peniculata* - Inoculation of *G. mosseae* was superior for cultivation of Kalmegh. Results revealed the best percentage root colonization (42 ± 1.07), AM spore number (251 ±1.12), root length (26.73 ± 1.15) and total dry biomass (22.91 ± 1.02) after 90 days of inoculation. Best results were obtained with *A. appendiculata* in terms of percentage root colonization (40 ± 1.09), AM spore number (218 ± 1.10) and total dry biomass (20.28 ± 1.09). Effect of *G. fasciculatum* and *A. appendiculata* were found similar in terms of root length (23.71 ± 1.51 and 22.87 ± 1.12 respectively).

All together, *G. mosseae* recorded its superiority with *Coleus aromaticus, Adathoda vasica* and *Andrographis peniculata;* however, inoculation of *A. appendiculata* was found superior for *Rauwolfia serpentina.*

Improvement of plant growth with inoculation of AM fungi has been reported in a number of plants including medicinal plants and attributed to the enhancement of nutrient absorption, via an increase in the absorbing surface area. A significant increase in percentage root colonization, spore number, root length and total dry biomass in all cases confirms the importance of AM inoculation for better establisment of these valuable plants. Sharma *et al.,* (2007) and Kumar *et al.,* (2008) studied the effects of *G. mosseae* and *A. laevis* alone and in combination with *T. viride* and reported effectiveness of *G. mosseae* for *Stevia rebaudiana* and *Spilanthes acmella.* Chiramel *et al.,* (2006), observed *G. leptotichum* and *G. intraradices* the two best AM symbionts for Kalmegh compared to others.

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Table 1 : Effect of AM fungi on growth of *Rauwolfia serpentina.*

S. No.	Treatment	% root colonization		AM spore number / 100 g soil		Root length (cm)		Total dry biomass (g)	
		45 D	90 D	45 D	90 D	45 D	90 D	45 D	90 D
1.	Control	11 ± 0.39	24 ± 0.45	27 ± 0.45	46 ± 0.35	23.5 ± 0.29	36.7 ± 0.32	28.9 ± 0.13	32.4 ± 0.30
2.	*Glomus aggregatum*	43 ± 0.33	76 ± 1.19	126 ± 1.14	354 ± 1.48	54.7 ± 1.39	74.2 ± 1.12	32.4 ± 1.23	44.6 ± 1.45
3.	*Glomus macrocarpum*	41 ± 1.44	76 ± 1.39	158 ± 1.24	280 ± 1.32	46.9 ± 1.43	56.5 ± 1.36	28.3 ± 1.12	41.2 ± 1.47
4.	*Glomus fasciculatum*	57 ± 1.59	70 ± 1.39	149 ± 1.35	245 ± 1.24	54.6 ± 1.01	67.2 ± 1.14	26.5 ± 1.07	33.5 ± 1.37
5.	*Glomus mosseae*	53 ± 1.35	78 ± 1.39	208 ± 1.15	328 ± 1.35	58.6 ± 1.33	71.8 ± 1.47	32.5 ± 1.09	49.7 ± 1.46
6.	*Acaulospora appendiculata*	48 ± 1.30	68 ± 1.36	210 ± 1.68	345 ± 1.18	55.2 ± 1.35	75.4 ± 1.82	34.2 ± 1.25	55.7 ± 1.27

± - Standard Deviation
45 D - After 45 days of inoculation
90 D - After 90 days of inoculation

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Table 2 : Effect of AM fungi on growth of *Adathoda vasica.*

S. No.	Treatment	% root colonization		AM spore number / 100 g soil		Root length (cm)		Total dry biomass (g)	
		45 D	90 D	45 D	90 D	45 D	90 D	45 D	90 D
1.	Control	07 ± 0.32	23 ± 0.13	14 ± 0.05	26 ± 0.30	18.4 ± 0.06	27.3 ± 0.03	14.35 ± 0.31	21.22 ± 0.33
2.	*Glomus aggregatum*	43 ± 1.3	56 ± 1.21	124 ± 1.07	247 ± 1.03	21.2 ± 1.05	29.9 ± 1.02	17.5 ± 1.04	28.5 ± 1.11
3.	*Glomus macrocarpum*	39 ± 1.53	68 ± 1.33	134 ± 1.43	219 ± 1.09	22.7 ± 1.05	34.6 ± 0.93	15.8 ± 1.09	26.2 ± 1.05
4.	*Glomus fasciculatum*	31 ± 1.37	57 ± 1.23	132 ± 1.26	246 ± 1.02	19.7 ± 1.25	30.5 ± 1.01	16.6 ± 1.21	26.9 ± 1.22
5.	*Glomus mosseae*	47 ± 1.23	78 ± 1.22	148 ± 1.33	320 ± 1.31	23.6 ± 1.26	40.5 ± 1.06	19.6 ± 120	32.8 ± 1.21
6.	*Acaulospora appendiculata*	32 ± 1.34	70 ± 1.41	142 ± 1.39	297 ± 1.26	22.9 ± 1.41	34.5 ± 1.11	16.8 ± 1.32	27.6 ± 1.51

± - Standard Deviation
45 D - After 45 days of inoculation
90 D - After 90 days of inoculation

Table 3 : Effect of AM fungi on growth of *Coleus aromaticus.*

S. No.	Treatment	% root colonization		AM spore number / 100 g soil		Root length (cm)		Total dry biomass (g)	
		45 D	90 D	45 D	90 D	45 D	90 D	45 D	90 D
1.	Control	07 ± 0.05	13 ± 0.08	32 ± 0.03	82 ± 0.31	06.56 ± 1.13	11.93 ± 1.21	15.87 ± 1.21	22.79 ± 1.03
2.	*Glomus aggregatum*	25 ± 1.21	33 ± 1.33	108 ± 1.22	173 ± 1.27	12.32 ± 1.04	20.45 ± 1.06	16.91 ± 1.31	25.71 ± 1.02
3.	*Glomus macrocarpum*	22 ± 1.09	32 ± 1.02	98 ± 1.20	178 ± 1.05	11.47 ± 1.01	20.58 ± 1.20	19.92 ± 1.05	25.80 ± 1.12
4.	*Glomus fasciculatum*	21 ± 1.04	43 ± 1.06	112 ± 1.12	189 ± 1.10	12.85 ± 1.09	22.81 ± 1.04	18.83 ± 1.01	23.93 ± 1.02
5.	*Glomus mosseae*	28 ± 0.30	58 ± 1.35	168 ± 1.24	245 ± 1.92	14.67 ± 1.31	27.56 ± 1.01	20.52 ± 1.02	35.93 ± 1.04
6.	*Acaulospora appendiculata*	27 ± 0.31	56 ± 0.21	127 ± 1.03	231 ± 1.05	12.70 ± 1.21	25.71 ± 1.20	18.93 ± 1.41	28.51 ± 1.13

± - Standard Deviation
45 D - After 45 days of inoculation
90 D - After 90 days of inoculation

Table 4 : Effect of AM fungi on growth of *Andrographis peniculata.*

S. No.	Treatment	% root colonization		AM spore number / 100 g soil		Root length (cm)		Total dry biomass (g)	
		45 D	90 D	45 D	90 D	45 D	90 D	45 D	90 D
1.	Control	05 ± 021	12 ± 0.04	23 ± 0.02	31 ± 0.51	06.23 ± 0.09	16.76 ± 0.06	08.23 ± 0.03	13.81 ± 0.05
2.	*Glomus aggregatum*	21 ± 1.03	37 ± 1.09	45 ± 1.31	104 ± 1.35	19.89 ± 1.31	11.44 ± 1.08	11.44 ± 1.08	19.11 ± 1.01
3.	*Glomus macrocarpum*	20 ± 1.36	39 ± 120	52 ± 1.21	112 ± 1.07	11.82 ± 1.22	21.84 ± 1.26	12.02 ± 1.07	19.72 ± 1.05
4.	*Glomus fasciculatum*	21 ± 1.45	35 ± 1.02	59 ± 1.22	125 ± 1.03	10.95 ± 1.25	23.71 ± 1.51	11.91 ± 1.02	19.47 ± 1.24
5.	*Glomus mosseae*	29 ± 1.61	42 ± 1.07	104 ± 1.30	251 ± 1.12	17.12 ± 1.08	26.73 ± 1.15	13.95 ± 1.01	22.91 ± 1.02
6.	*Acaulospora appendiculata*	26 ± 1.27	40 ± 1.09	94 ± 1.21	218 ± 1.10	15.84 ± 1.29	22.87 ± 1.12	12.71 ± 1.07	20.28 ± 1.09

± - Standard Deviation
45 D - After 45 days of inoculation
90 D - After 90 days of inoculation

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A Comparative Study of Two *Rhizobium* Isolates from *Crotolaria juncea* L. and *Vigna radiata* L.

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In the present work Rhizobia were isolated from the root nodules of two plants of *Crotolaria juncea* (L.) and *Vigna radiata* (L.) which belong to family Papilionaceae (Leguminosae), by adapting the standard microbiological procedures. They were grown in Norris and Date (YEM) Broth and purified by dilution techniques and again they were freshly inoculated on YEM broth and kept for 24 hours to a week. Their optical density (OD) were measured spectrophotometrically at 660 nm on different incubation periods from 30 minutes up to 25 hours. It has been observed that after 16 - 18 h of incubation, their was an enhancement (peak period) of Rhizobia in both Rhizobia samples. It was also observed that the growth declination starts after 20 hours in all the Rhizobia of their respective species.

Keywords : Rhizobia, *Crotolaria juncea* (L.), *Vigna radiata* (L.), Root nodules, Growth culture.

INTRODUCTION

Now-a-days the root nodule Rhizobia are used as one of the biofertilizers in legumes (Fred *et al.,* 1967) to increase in crop yield as well as to cut the price of chemical fertilizers. To increase the crop production, cut short of chemical prices and to retain the soil fertility for a longer period, these bio-fertilizers are very useful source for farmers (Burton, 1967). The information about the effectivity of Rhizobia are less available. Dey and Srivastava (2002) also reported the biodiversity of the *Rhizobium* species in respect to their growth and nitrogen fixing efficiency in free living cultures. Therefore, the present work was undertaken and planned to know the effectivity of Rhizobia during the year 2001-2002.

MATERIAL AND METHODS

An experiment was conducted in the Research Laboratory of Dept. of Botany, P.G.T.D., R.T.M., Nagpur University, Nagpur during the year 2001-2002.The root nodules of *Crotolaria juncea* (L.) and *Vigna radiata* (L.) were collected, washed thoroughly, sterilized with sterilent $HgCl_2$ 0.1% (Motsara *et al.*, 2001) and inoculated on Norris and Date (1976) YEM Agar medium. After a three-four days they were transferred to new media of same YEM broth and purified by serial dilution. Again they were broth cultured sequentially in a series of test tubes and their optical density was measured spectrophotometrically at 660 nm from 30 minutes to 25 hours.

RESULTS AND DISCUSSION

The *Rhizobium* species of *Crotolaria juncea* (L.) and *Vigna radiata* (L.) shows the enhancement period of growth at 16-18 hours. The Rhizobia of *Crotolaria juncea* (L.) showed an enhancement at 17 h of incubation where as of *Vigna radiata* (L.) was at 16.5 h (Ierusalimskii,1967). It has been observed that in another species of Rhizobia the condition was also same and they showed differed growth peaks. The results were in line with Dey and Srivastava (2002). Therefore, it was concluded that the *Rhizobia* species which are slow growing shows different growth patterns in broth cultures.

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Observation Table (1) : Optical Density of *Crotolaria juncea* (L.) and *Vigna radiata* (L.)

Duration in hours	*Vigna radiata*	*Crotolaria juncea*	Duration in hours	*Vigna radiata*	*Crotolaria juncea*
½	.046	.044	14	.860	.385
1	.095	.063	14½	.865	.401
1½	.143	.097	15	.902	.418
2	.191	.098	15½	.954	.436
2½	.223	.103	16	1.028	.452
3	.240	.113	16½	1.095	.495
3½	.288	.125	17	1.091	.525
4	.301	.135	17½	1.090	.525
4½	.345	.143	18	.968	.524
5	.391	.160	18½	.929	.520
5½	.395	.173	19	.901	.501
6	.402	.185	19½	.883	.490
6½	.421	.200	20	.865	.475
7	.450	.209	20½	.853	.462
7½	.472	.223	21	.842	.455
8	.495	.235	21½	.837	.441
8½	.507	.249	22	.823	.430
9	.560	.257	22½	.813	.421
9½	.579	.270	23	.798	.412
10	.623	.282	23½	.775	.400
10½	.624	.296	24	.769	.395
11	.685	.308	24½	.745	.386
11½	.787	.315	25	.722	.360
12	.802	.339			
12½	.833	.347			
13	.835	.357			
13½	.850	.370			

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Management of Wilt and Root Rot o Safflower Through Biogents

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A field experiment was conducted in farmers fields to see the efficacy of six fungal biogents combined with *Pseudomonas fluorescens*. Expect *Aspergillus fumigatus* + *P. fluorescens* all the other biogents reduced seedling mortality significantly as compared to control. Amongst biogents both the species of *Trichoderma* and *Gliocladium virens* combined with, *P. fluorescens* were more promising than other combination of biogents. These were also effective in reducing wilt, root rot or their combined infection. Maximum grain yield was obtained in *T. viride* + *P. fluorescens* combinations.

Keywords : Seedling mortality, Biogents, Grain yield.

INTRODUCTION

Wilt (*Fusarium oxysporum* f. sp. *carthami*) and root rot (*Macrophomina phaseolina*) are the soil borne major diseases of safflower taking heavy toll of the safflower crop. The problem is so severe that growers from Hingoli district and problematic area of Parbhani district (Maharashtra State) have replaced this crop with other crop. Suitable management strategies are not available.Also resistant varieties for these diseases are still in the evaluation process. Under such circumstances farmers face huge loss every year. Therefore, in present study previously tested and promising biogents have been evaluated for their efficacy in managing wilt and root rot of safflower in infested field.

MATERIAL AND METHODS

A field experiment was conducted in randomized block design with three replications in village Kausadi Dist- Parbhani (M.S.) in farmer's field where wilt and root rot were noticed in earlier years. Carrier based inoculants of six fungal bio-agents (*Trichoderma viride, T. harzianum, Gliocladium virens, Aspergillus niger, A. fumigatus* and *Paecilomyces varioti*) combined separately with one bacterial biogent (*Pseudomonas fluorescens*) were applied to seeds of cultivar Neera at the rate of 4g / Kg (2g fungal biogent + 2g bacterial biogent) at the time of sowing. These biogents were studied on the basis of their performance in *in vitro* study.Gross and net plot sizes were 4 x 2.25 m² and 3.10 x 1.45 m², respectively.The crop was sown at the distance of 45 x 20 cm². Fertilizer @ 30 Kg N + 40 Kg P_2O_5 per ha was applied at the time of sowing and crop was top dressed with 30 Kg N/ha at 35 days after sowing. The crop was irrigated for four times including presowing irrigation. The experimental field was kept weed free by regular hand weedings. Foliar pests were controlled by dusting and spraying recommended insecticides. Mortality of safflower was counted at 30,60 and 90 days after sowing. On the basis symptoms and microscopic observations of cross sections roots the dead seedlings were categorized into wilted, root affected and seedlings showing infection of both (wilt and rc rot).

RESULTS AND DISCUSSION

It is evident from the results that expect *Aspergill fumigatus* + *Pseudomonas fluorescens* all the other bioger reduced seedling mortality significantly as compared to cont (Table 1). In general both the species of *Trichoderma* a *Gliocladium virens* combined with *P. fluorescens* were mc promising than other combinations of biogents. Reduction safflower mortality due to biogents over control varied frc 28.31 per cent to 74.96 per cent at 90 days after sowing. management of individual diseases expect *A. fumigatus* + *fluorescens* all the remaining biogents significantly decreas wilt as compared to control. Minimum wilt incidence w recorded in both the species of *Trichoderma* combined w *P. fluorescens* which was significantly less than rest of tl treatments (Table 2). As regards to root rot minimum inciden was observed in both the species of *Trichoderma* combin with *P. fluorescens* which was at per with *G. virens* + *fluorescens* and significantly less than other biogents a control. In combined infection of wilt and root rot both tl species of *Trichoderma* and *G. virens* combined with *fluorescens* were at per with *P. varioti* and *P. fluorescens* a reduced disease significantly as compared to control.

As regards to seed yield per hectare maximum yie was obtained in *T. viride* + *P. fluorescens* which w significantly more than *A. niger* and *A. fumigatus* combin with *P. fluorescens*. It was followed by *T. harzianum* and *virens* in combination with *P. fluorescens* which has significan higher yield than *A. fumigatus* + *P. fluorescens*. Increase seed yield over control in biogents varied from 25.08 per ce

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able 1 : Mortality (%) of safflower as influenced by seed treatment with biogents at Kausadi location.

r. o.	Treatments	Mean Mortality(%)			Per cent decrease over control	Yield (Kg/ ha)	Per cent increase over control
		Days after sowing					
		30	60	90			
.	*T. viride + P. fluorescens*	1.91 (7.84)	6.08 (14.26)	8.78 (17.21)	63.84	1123	59.74
.	*T. harzianum + P. fluorescens*	1.51 (6.95)	5.34 (13.20)	7.59 (15.96)	68.74	1104	57.04
.	*G. virens + P. fluorescens*	2.74 (9.46)	7.46 (15.76)	9.69 (18.13)	60.09	1078	53.34
.	*A. nigar + P. fluorescens*	5.81 (13.74)	10.64 (18.93)	15.83 (23.43)	34.80	948	34.85
.	*A. fumigates + P. fluorescens*	4.91 (12.69)	12.60 (20.73)	17.89 (24.96)	26.32	879	25.04
.	*P. varioti + P. fluorescens*	5.58 (13.58)	9.94 (18.35)	14.80 (22.54)	39.04	984	39.97
.	Untreated control	8.55 (16.91)	15.78 (23.38)	24.28 (29.50)	-	703	-
	S. E. +	1.02	1.06	0.76	-	56	-
	C .D. at 5 %	3.13	3.27	2.33	-	174	-

igures in parentheses are arcsine values and statistics was applied to them.

able 2 : Wilt and root rot (%) of safflower as influenced by seed treatment with biogents in field at Kausadi location.

r. o.	Treatments	Mean Mortality (%) (90 days after sowing)			Per cent decrease over control		
		Wilt	Root rot	Wilt + root rot	Wilt	Root rot	Wilt + root rot
	T. viride + P. fluorescens	4.16 (11.76)	2.70 (9.38)	1.91 (7.84)	59.38	66.62	67.79
	T. harzianum + P. fluorescens	3.40 (10.62)	2.26 (8.64)	1.82 (7.82)	66.80	72.06	69.3
	G. virens + P. fluorescens	3.91 (11.20)	3.84 (11.20)	1.92 (7.89)	61.81	52.53	67.62
	A. nigar + P. fluorescens	6.06 (14.21)	5.20 (13.14)	4.56 (12.17)	40.82	35.72	23.10
	A. fumigates + P. fluorescens	8.55 (16.93)	4.91 (12.69)	4.41 (12.11)	16.50	39.30	25.63
	P. varioti + P. fluorescens	5.98 (14.08)	5.60 (13.63)	3.21 (10.24)	41.60	30.78	45.87
	Untreated control	10.24 (18.62)	8.09 (16.50)	5.93 (13.99)	-	-	-

gures in parentheses are arcsine values and statistics was applied to them.

. fumigatus + P. fluorescens) to 59.74 per cent (*T.viride + P. uorescens*).

Usefulness of *T. viride + P. fluorescens* in reducing wilt nd root rot of different crops has been reported by various orkers (Ushamalani *et.al* 1997; Gholve and Kurundkar,2002; ehere *et al* 2002; Prasad, 2003 and Sajeena *et al.* 2003). hese observations support findings of present study. As gards to *T. harzianum + P. fluorescens,* Singh (2000) reported fectiveness of this fungus in the management of *Fusarium dum*. Also Behere *et al* (2002) reported effectiveness of this ngus in managing root rot caused by *M. phaseolina*. These bservations are in agreement with the observations of present udy. Usefulness of *G. virens* in managing seedling mortality ue to wilt and root rot diseases observed in present study is similar line with those reported by Yadav and Tripathi (1999), ukharjee and Tripathi (2000),Desai and Kulkarni (2002), wari and Mukhopadhyay(2001) and Behere *et al* (2002). crease in grain yield due to biogents *T. viride, T. harzianum* nd *G. virens* has been reported by several workers (Mukharjee nd Tripathi ,2000; Granada *et al*,1999; and Sajeena *et al*, 03). Observations of present study are in agreement with e same.

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Pathogenic Potential of Seed Mycoflora of Spinach (*Spinacea oleracea* L.)

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Using blotter and agar plate methods as recommended by ISTA, the seed borne mycoflora of 20 spinach seed sample collected from different areas of Osmanabad district of Maharashtra, India were examined. A total number of 12 species belonging to 9 genera of fungi were isolated. Saprophytic fungi were pre-dominantly present. The germination of seeds was markedly suppressed by all fungi. Maximum reduction in germination was observed in the seeds inoculated with *C. lunata* and *B. cinarea*, whereas maximum decrease in plumule and redical length was observed in *C. lunata*. The maximum inhibition of germination, plumule and radicle length were observed with soil inoculated with *A. flavus*. Culture filtrates of all the associated fungi caused reduction in germination, plumule and radicle length.

Keywords : Seed mycoflora, Spinach, Soil inoculation method, Seed inoculation method, Cultrate filtrates.

INTRODUCTION

Spinach (*Spinacea oleracea* L.) is an economically important vegetable crop. Although many diseases have been reported as externally and internally seed borne (Farr *et al.*, 1989, Summer, 1991, Walkar, 1952, Correll *et al.*, 1994. Bhale, 2002 and Raid & Kucharek, 2003), which causes discoloration, spoilage of seeds, germination effects, rotting and wilting of seedlings, stem gall, blight, leaf spot, powdery mildew, downy mildew, rust and anthracnose. Crop loss due to these diseases is substantial as it leads to capital losses upto 20 to 60%. The number of seed borne fungi and their incidence was reduced by pretreatment with mercuric chloride. It was indicated that fungi present even after pretreatment were internally seed borne and were not surface contaminants (Abdus Shakir & Sultan, 2000). Understanding the nature of spinach seed infection by these pathogens may help to determine the potential for seed transmission, as well as the potential efficacy of chlorine, hot water of fungicide seed treatment for eradication of the fungi from infected seed/ or prevention of seed transmission. In the present investigations, an attempts was made to observed the effect of seed mycoflora and their culture filtrate on germination and seedling vigour of spinach.

MATERIAL AND METHODS

A total 20 spinach (*Olive green* var.) seed sample were assayed during this study. Seed samples were collected from farmer fields and markets of Osmanabad district of Maharashtra. The seeds were collected in sterilized cloth bags. Detection of internal and external seed borne fungi was carried out by the standard blotter technique and agar plate method (ISTA, 1976).

Screening of seeds for associated mycoflora : The incubated seeds were screened on 7th day by using compound microscope. The fungi on the seeds were identified by observing habit characteristics features. The associated mycoflora were recorded and identified with the help of standard manuals (Barnett, 1960, Ellis, 1971, Subramanian, 1971 and Mukadam, 1997).

Seed inoculation Technique : Spinach seeds were surface sterilized with 0.1% $HgCl_2$ solution for 2 min followed by three washing with sterile distilled water and inoculated by 7th day old sporulating culture of each fungus. The seeds were shade dried for 24 h at room temperature 28 ± 2ºC. Ten inoculated seeds were sown separately in 30 cm earthen pots. After 10 days of sowing, observations on seed germination, radical and plumule length of seedlings were recorded.

Soil inoculation technique : The isolated fungi were grown separately on autoclaved rice medium (10 g rice and 5 ml distilled water) in 100 ml conical flasks. The flasks were incubated at 28 ± 2ºC for 10 days. Sterilized earthen pots (30 cm) were filled with pre-sterilized soil for inoculation; the upper 4 cm laye of soil was thoroughly mixed with rice medium for supporting the fungal growth. The pots were covered with sterilized polythene bags and left 24h in pot house. After 24 h of inoculation, 10 sterilized seeds of spinach were sown in each pot. Equal number of surface sterilized seeds sown in uninoculated soil served as a control. After 10 days, observations on seed germination, plumule and radicle length were recorded.

Effect of culture filtrate : The isolated fungi were grown in Richard's broth medium and incubated for 21 d at temperature 28 ± 2ºC. Culture filtrates were obtained by filtering the contents

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Table 1 : Effect of Seed inoculation technique of seed borne fungi on spinach.

Mycoflora	Seed germination (%)	Plumule (mm)	Radical (mm)
Aspergillus flavus	75 (88.98)	21 (22.46)	14 (14.65)
Aspergillus niger	83 (96.70)	31 (32.97)	20 (21.33)
Aspergillus fumigatus	73 (87.12)	19 (20.20)	13 (13.56)
Alternaria alternata	71 (84.40)	18 (19.08)	12 (12.47)
Curvularia lunata	55 (64.28)	13(13.56)	10 (10.32)
Bipolaris tetramera	63 (74.52)	15 (15.75)	19 (20.20)
Botrytis cinarea	57 (66.85)	13 (13.56)	11 (11.39)
Fusarium solani	63 (74.520)	18 (19.08)	13 (13.56)
Fusarium oxysporum	64 (75.78)	18 (19.08)	13 (13.56)
Rhizopus stolinifer	85 (98.12)	37 (41.47)	24 (25.91)
Penicillum notatum	65 (77.04)	19 (20.20)	14 (14.65)
Ulocladium sp	69 (81.98)	41 (46.44)	23 (24.77)
Control	89 (99.87)	41 (46.44)	32 (35.37)
CD (P=0.05)	6.39	6.24	3.89
CV (%)	14.54	42.42	36.93

Figures in parentheses are arcsine transformed value.

Table 2 : Effect of soil inoculation technique of seed borne fungi on spinach.

Mycoflora	Seed germination (%)	Plumule (mm)	Radical (mm)
Aspergillus flavus	42 (47.69)	14 (14.65)	11 (11.39)
Aspergillus niger	72 (85.55)	31 (32.97)	23 (24.77)
Aspergillus fumigatus	73 (87.12)	32 (35.37)	24 (25.91)
Alternaria alternata	77 (91.12)	29 (31.78)	23 (24.77)
Curvularia lunata	80 (94.12)	41 (46.44)	32 (35.37)
Bipolaris tetramera	44 (50.21)	14 (14.65)	12 (12.47)
Botrytis cinarea	47 (54.02)	15 (15.75)	12.5 (13.00)
Fusarium solani	49 (56.57)	16 (16.85)	13 (13.56)
Fusarium oxysporum	45 (51.48)	16 (16.85)	13. (13.56)
Rhizopus stolinifer	79 (93.15)	34 (37.79)	31 (32.97)
Penicillum notatum	70 (83.19)	29 (31.78)	22 (23.61)
Ulocladium sp	58 (68.13)	18 (19.08)	15 (15.75)
Control	85 (98.12)	42 (47.69)	33 (36.58)
CD (P=0.05)	9.65	6.27	4.61
CV (%)	24.28	39.26	39.19

Figures in parentheses are arcsine transformed value.

through Whatmann filter paper No.1. Spinach seeds were separately soaked in these culture filtrates for 12 h and allowed to germinate in sterilized plates containing moist sterile blotter paper. Seeds soaked in uninoculated sterilized medium and sterile water served as control. Petri plates were incubated at 28 ± 2°C for 10 days. After incubation period, per cent seed germination and seedling vigour (Baki and Anderson, 1973) were recorded.

RESULTS AND DISCUSSION

The germination of seeds was markedly reduced by all fungi *viz.*, *A. flavus*, *A. niger*, *A. fumigatus*, *A. alternata*, *C. lunata*, *B. tetramera*, *B. cinarea*, *F. solani*, *F. oxysporum*, *R. stolonifer*, *P. notatum* and *Ulocladium* sp. Maximum reduction in germination over control was observed in the seeds inoculated with *C. lunata* and *B. tetramera*. Maximum decrease in plumule and radicle length was observed in *C. lunata*, whereas *A. niger* and *R. stolonifer* did not effect on plumule and radicle length (Table 1). The maximum inhibition of

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Table 3 : Effect of culture filtrate of seed borne fungi on spinach.

Mycoflora	Seed germination (%)	Plumule (mm)	Radical (mm)
Aspergillus flavus	12 (12.47)	7 (8.36)	6 (7.57)
Aspergillus niger	20 (21.33)	12 (12.47)	10 (10.32)
Aspergillus fumigatus	24 (25.91)	14 (14.65)	11.5 (11.93)
Alternaria alternata	67 (79.53)	28 (30.59)	19 (20.20)
Curvularia lunata	46 (52.75)	20 (21.33)	16 (16.85)
Bipolaris tetramera	51 (59.13)	24 (25.91)	17 (17.96)
Botrytis cinarea	54 (62.99)	25 (27.07)	20 (21.33)
Fusarium solani	47 (54.02)	21 (22.46)	18 (19.08)
Fusarium oxysporum	48 (55.29)	21 (22.46)	19 (20.20)
Rhizopus stolinifer	26 (28.24)	16 (16.85)	12 (12.47)
Penicillum notatum	29 (31.78)	18 (19.08)	14 (14.65)
Ulocladium sp	33 (36.58)	19 (20.20)	16.5 (17.40)
Control	91 (99.95)	39 (43.94)	36 (40.24)
CD (P=0.05)	12.99	4.80	4.31
CV(%)	19.51	36.53	41.49

Figures in parentheses are arcsine transformed value.

germination was observed in soil inoculation (Table 2) with *A. flavus*, followed by *B. tetramera* and *F. oxysporum*. Maximum decrease in plumule and radicle length was observed due to *A. flavus* treatment as compared to control. Culture filtrates of all the fungi caused reduction in seed germination; plumule and radicle length (Table 3). Results revealed that maximum inhibition in germination was recorded with the culture filtrate *A. flavus*, whereas *A. alternata* culture filtrate was less inhibitory as compared to control.

The findings of earlier workers also indicates that fungi reduced the per cent seed germination and seedling vigour (Everts, 2001 and Fluentes, 1988). Conditions favorable for long term storage of seed of most crops include 4 to 10% relative humidity (Neergard, 1977). Saprophytic fungi *viz., A. flavus* and *A. niger* were predominant among the fungi isolated reports have been made by Dawar and Ghaffar (1991) on sunflower seed, Mukherjee *et al.*, 1992 and Rasheed *et al.*, (2004) on groundnut seed and on soybean seed (Tariq *et al.*, 2005). Samota and Singh (2006) revealed that maximum inhibition in germination was recorded with the culture filtrate of *Aspirgillus flavus* and *Alternaria alternata* as compared to other fungi but was less inhibitory as compared to control. Surface sterilization of seed reduced the infection of *A. flavus* and *A niger* and increased the incidence of pathogenic fungi viz., *M. phaseolina*, *R. solani* and *F. moniliforme* on chickpea seed (Dawar *et al.*, 2007). Hernandez-Perez and Toit (2006) reported the greater potential difficulty at eradicating *Stemphyllum botryosum* and *Cladosporium variabile* from infected spinach seed using seed treatments.

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Rate of Spread of Yellow Vein Mosaic Disease as Influenced by Different Mulching in Okra (*Abelmoschus esculentus* (L.) Moench)

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Experiment were conducted to check rate of spread of yellow vein mosaic disease of Okra by repelling the whiteflies through different polythene mulching and wheat straw mulching indicated that the yellow polythene mulching stood first in *Kharif* and wheat straw mulching in summer season. In *Kharif* the yellow polythene mulching show less infection rate upto 45 to 60 days after sowing than other polythene mulching, while in summer wheat straw mulching show lower infection rate upto 45 to 60 days after sowing while in other mulching infection was early and highest infection rate was observed at 30 to 45 days after sowing. Wheat straw, yellow polythene and blue polythene mulching were effective to make the progress of the disease slower than the rest of the mulches in both *Kharif* and summer seasons.

Keywords : Okra, Yellow Vein Mosaic Virus, Whitefly, Infection rate, Mulching.

INTRODUCTION

Yellow vein mosaic (YVM) disease showed characteristics symptoms as veinal chlorosis, yellowing, stunting, reduction in the number of leaves, reduced size and weight of fruit and fruit yield. The yellow vein mosaic virus disease is an age old problem of okra cultivation in this country. Although disease was reported as early 1924 (Kulkarni 1924). The transmission characteristic and epidemiological aspect of the virus was studied by several workers (Uppal *et al.*, 1940; Capoor and Verma, 1950). The pesticidal control of the spread of the disease has also been studied by many workers (Srabani Debnath *et al.*, 2002 and Shivpuri *et al.*, 2004). Also cultural methods to control of spread of disease was studied by Khan and Mukhopadhyay (1985) but the spread of disease influenced by different mulching was not yet studied so the present investigation was carried out.

MATERIAL AND METHODS

To study the effect of mulches on infection rate and disease progress a field experiment was conducted. The experiment was laid out in randomized block design having six treatments with four replications. The sowing was done on 30th July 2005 in *Kharif* season and 16th February, 2005 in summer season using the seeds of cultivar Parbhani Kranti. The crop was cultivated by adopting all recommended cultural practices. The mulching in the plot was done as per treatment. The plots were exposed to natural invasion by whiteflies to develop the disease.

Whitefly count was recorded as per the method modified from Sipell *et al.* (1982).

The plants showing infection to yellow vein mosaic were marked at different growth stages *i.e.*, from incidence to 120 days after sowing with and interval of 15 days. The percentage of incidence was worked out.

An apparent infection rate (r) as influenced by different treatments in the experiment was calculated by using the formula as suggested by Van der Plank (1963).

$$r = \frac{2.3}{t_2 - t_1} \log_{10} \frac{x_2 (1 - x_1)}{x_1 (1 - x_2)}$$

Where,

r = Infection rate in unit per day.

$t_2 - t_1$ = Time (days) between first observation and subsequent observation.

x_1 and x_2 = Percentage (% /100) of disease on the first and subsequent observation dates, respectively.

Similarly, based on incidence at different stages of the crop, the disease progress curves were drawn as cumulative curves by plotting disease value as $\log_e (1/1-x)$ against time (Van der Plank, 1963).

RESULTS AND DISCUSSION

The data on infection rate as influenced by different coloured polythene mulches and wheat straw mulches is presented in Table 1.

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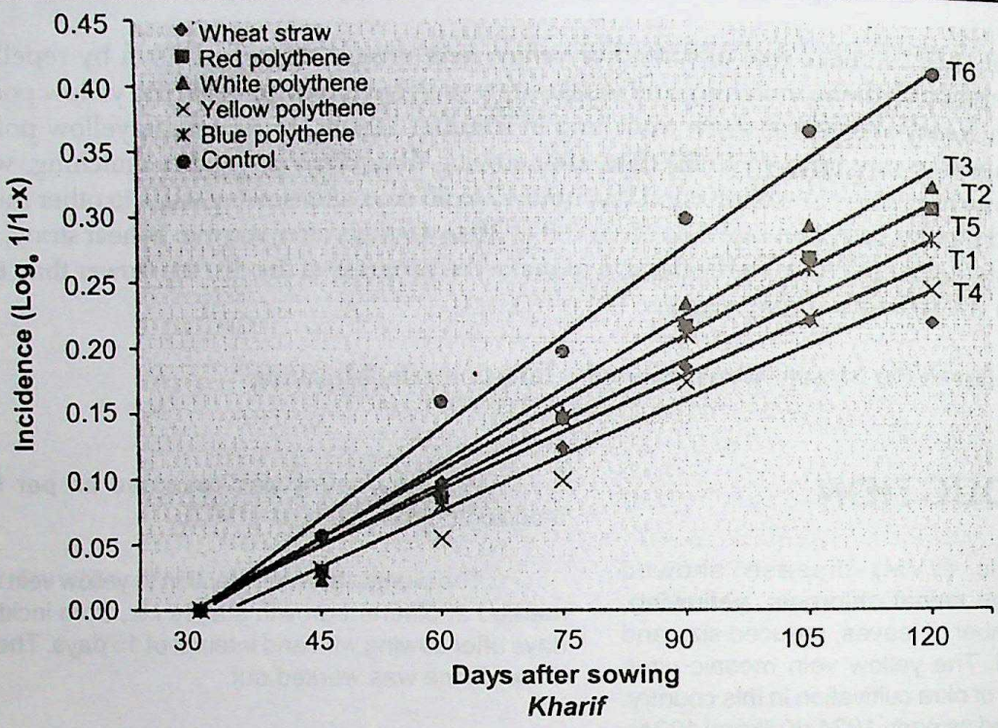
Table 1 : Influence of various mulches on YVM disease development (r) in okra Cv. Parbhani Kranti.

Treatments	Apparent infection rate (r*) at different growth development stages													
	Kharif							Summer						
	15-30	30-45	45-60	60-75	75-90	90-105	105-120	15-30	30-45	45-60	60-75	75-90	90-105	105-120
Wheat straw (T_1)	0.0	0.0	0.082	0.016	0.028	0.012	0.0	0.0	0.0	0.091	0.037	0.039	0.013	0.004
Red polythene (T_2)	0.0	0.0	0.071	0.037	0.028	0.016	0.010	0.0	0.139	0.065	0.053	0.034	0.010	0.003
White polythene (T_3)	0.0	0.0	0.097	0.030	0.032	0.017	0.007	0.00	0.085	0.066	0.050	0.027	0.019	0.007
Yellow polythene (T_4)	0.0	0.0	0.040	0.041	0.039	0.018	0.007	0.0	0.075	0.057	0.042	0.020	0.010	0.003
Blue polythene (T_5)	0.0	0.0	0.066	0.041	0.025	0.016	0.006	0.00	0.103	0.070	0.037	0.020	0.011	0.006
Control (T_6)	0.0	0.0	0.072	0.015	0.031	0.016	0.009	0.00	0.055	0.049	0.44	0.032	0.017	0.005



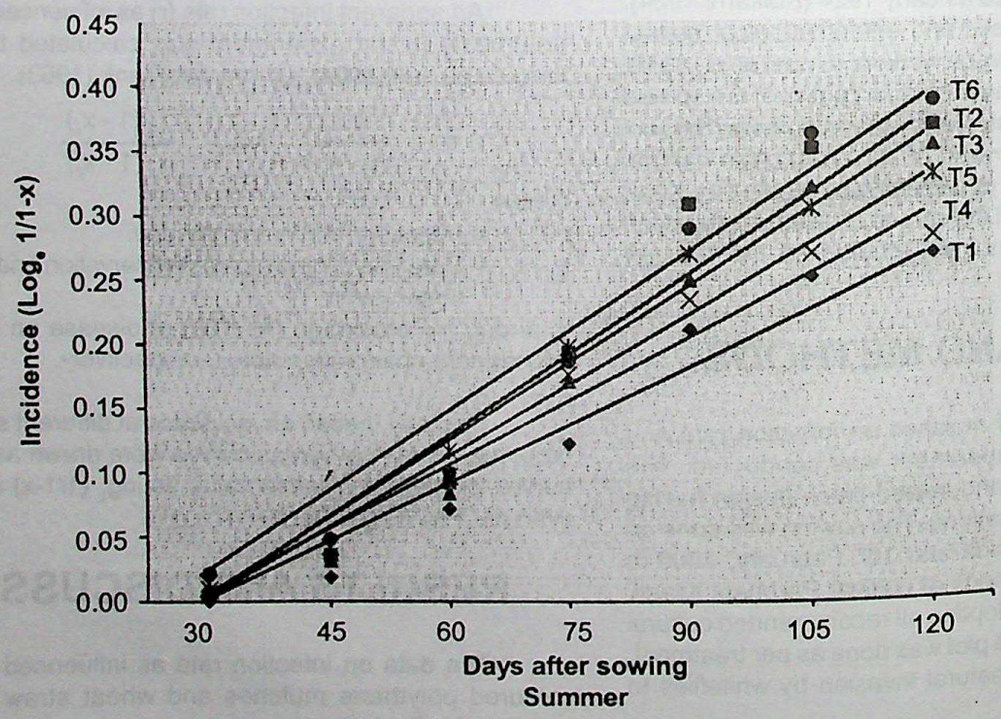


Fig. 1. Yellow vein mosaic disease progress in okra influence by different mulches.

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The data revealed that, the incidence started 30 and 15 days after sowing in *Kharif* and summer, respectively. The different mulches were found not to influence the infection of the okra by yellow vein mosaic virus in both *Kharif* and summer seasons. As in *Kharif*, the infection occurred after 30 days after sowing, the infection rates were found to be more between 45 to 60 days after sowing in all the mulches and at subsequent growth stages the infection rate decreased progressively with increase in age of the crop. In summer season, occurrence of the disease was noticed earlier than the *kharif*. In summer the disease incidence was noticed at 15 days after sowing with exception of mulching with wheat straw. The mulching with wheat straw delayed the disease occurrence by 15 days than the polythene mulches. However, the mulches were found not to have marked influence on the progress of the disease. The higher infections in polythene mulches were recoded between 30 to 45 days after sowing and in wheat straw between 45 to 60 days after sowing. In subsequent growth stages in all the mulches, the infection rate decreased progressively with increase in the age of crop.

The progress of yellow vein mosaic of okra as influenced by different coloured polythene and wheat straw mulches is depicted in Fig. 1.

All the coloured polythene and wheat straw mulches exhibited slower progress of the disease than control in both *kharif* and summer seasons. The progress of the disease in all the mulches including control comparatively was slower in *kharif* than summer. The three mulches *viz.*, wheat straw (T_1), yellow polythene (T_4) and blue polythene (T_5) were effective to make the progress of the disease slower than rest of mulches in both *kharif* and summer seasons. In this respect, yellow polythene stood first in *kharif* and wheat straw in summer.

The effect of red, white, yellow and blue polythene and wheat straw mulches on infection rate was studied. The wheat straw mulch was found to cause delay in infection in summer upto 30 days after sowing. In all the mulches at seedling stage between 45 to 60 days after sowing in *Kharif*, the infections were more and decreased in subsequent growth stages. The yellow polythene mulch had lowest infection rate than other mulches at 45 to 60 and 30 to 45 days after sowing in *Kharif* and summer, respectively. Similar results regarding incidence of yellow vein mosaic was reported by Khan and Mukhopadhyay (1975). They reported that yellow polythene mulch significantly delayed and reduced the incidence of yellow vein mosaic of okra. Of the tested mulches, yellow polythene exhibited slow development of the disease than rest of the mulches in *Kharif* and wheat straw in summer season. Similar results in respect to effect of yellow polythene mulch on incidence and progress of the disease has been reported by Khan and Mukhopadhay (1975).

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Quantitative Relationship Between Leaf Rust Disease Severity and Leaf Yield Loss in Mulberry

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Foliar diseases significantly reduce the mulberry (*Morus alba* L.) yield both quality and quantity. Among the diseases, leaf rust caused by *Cerotelium fici* constitutes the greatest epidemiological constraint causing considerable damage in leaf productivity in different agro climate zones. An experiment was under taken in three consecutive years (2006, 2007 and 2008) to estimate the leaf yield loss in relation to leaf rust severity in summer, rainy and winter seasons in seven promising mulberry cultivars (Local , K-2, S-13, S-34, S-36, S-54 and MR-2). The mean leaf yield loss varied from 2.69 to 22.90% in rainy and 3.19 to 29.07% in winter season in different mulberry cultivars. The leaf yield loss was maximum in S-36 (29.07%) followed by S-13 (20.43%) and K-2 (20.40%) in winter season. K-2 and S-13 have shown similar trend of disease severity in rainy season, where as S-36 has shown considerably less PDI. Regression analysis was conducted to study the relationship between leaf rust severity and leaf yield loss in different mulberry cultivars and pooled data. Yield loss model indicated that there was a definite linearity between leaf rust severity and yield loss by showing high co-efficient determination (R^2) more than 90%. The derived yield loss model was Y= –2.95 + 0.85** (rainy) Y= –2.73 + 0.92** (winter). Based on the results of the present investigation the cultivars MR-2, S-34, S-54 can be advocated as they are considered as slow rusters for cultivation in agroclimatic zones where longer duration of precipitation prevails. These cultivars may be exploited in breeding programme to increase the level of rust resistance.

Keywords : Leaf rust; *Cerotelium fici;* Severity; Leaf yield loss; Mulberry.

INTRODUCTION

Mulberry (*Morus alba* L.) of the family Moraceae is one of the important commercial crop grown under wide range of climatic conditions and play a pivotal role in silk production and intern on the economic development of the country. The leaf yield potential is varied in different mulberry cultivars. Foliar diseases significantly reduce the yield both quality and quantity. Among the different foliar diseases (leaf spot, leaf blight, powdery mildew) leaf rust caused by *Cerotelium fici* (Artho) constitutes the major epidemiological constraint affecting almost all the mulberry cultivars cultivating in South India (Gunasekhar *et al.*, 1994). Characteristic symptom of this disease included the appearance of reddish brown pinhead pustules on the abaxial surface of leaf leaving small brownish spots on the adoxial surface leading to yellowing drying and premature defoliation. The importance of the disease has increased in recent years on the extensive cultivation of mulberry in different agro climatic zones. The disease on set and its progress in greatly influenced by the environmental factors, crop age and host susceptibility (Srikantaswamy *et al.*, 2006). Besides the farmers are highly heterogeneous in their brushing programmes, leading to the non uniform set of leaf harvest resulting in the availability of different age groups of leaves for the rust pathogen multiplication round the year. When the congenial conditions prevail the intensity of disease is very high causing maximum crop loss. Literature survey revealed that most of the studies dealt with chemical control, nutrition changes, yield losses and epidemiological factors on disease onsets and its progress. However, the relationship between the leaf yield loss in relation to disease severity was not known in different cultivars, which are in field. Hence, the experiment was undertaken for a period of three years (2006-2007 and 2008) in three cropping seasons (Summer, rainy and winter) to quantify the relationship between the leaf rust severity and leaf yield loss and to develop yield loss model.

MATERIAL AND METHODS

Establishment of mulberry plantation : Apparently disease free cuttings of promising mulberry cultivars such as Local, K-2, S-13, S-34, S-36, S-54 and MR-2 were collected from mulberry germplasm of CSRTI, Mysore. Three months old saplings raised in nursery were transplanted under 60 cm x 60 cm spacing in five replication accommodating 100 plants replication/micro-plot leaving guard row all around in Randomized Block Design. Recommended agronomical package of practices were followed *i e.*, weeding, irrigation, application of organic fertilizers (FYM) at 20 metric tones/ha/yr in two split doses and inorganic fertilizers at 300N, 120P and 120K in five equal split doses during the period of experiment to avoid any physiological stress in plant system.

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Seasonal influence of PDI of leaf rust in promising mulberry cultivars : The percentage of disease index (PDI) was determined in seven promising mulberry cultivars for different seasons like summer, rainy and winter in 2006, 2007 and 2008. PDI was determined at the leaf harvest stage of the crop. During summer season, the crop was evaluated from Mar - May, in rainy season from Jul - Sept. and in winter season from Nov - Jan. PDI was recorded by following 0 - 4 scale (Anon, 1984).

0 grade	=	No disease symptoms
1 grade	=	Up to 5% of leaf area affected
2nd grade	=	5.1 - 20% leaf area affected
3rd grade	=	20.1 - 50% leaf area affected
4th grade	=	50.1 - 100% leaf area affected

After arriving at the grade of infection, the per cent disease index (PDI) was calculated by following the formula.

$$PDI = \frac{\text{Sum of numerical value}}{\text{Total No. of leaves graded X Max. grade (}i\,e.\text{,4)}} \times 100$$

(The numerical values were obtained by multiplying the number of leaves with their respective grading)

Based on the disease reaction the cultivars were identified as resistant/susceptible following the disease scale.

0.1 - 5% of PDI	=	Resistant
5.1 - 20% of PDI	=	Moderately Resistant
20.1 - 50% of PDI	=	Susceptible
Above 5% of PDI	=	Highly susceptible

Data were collected for three years in each season from seven promising mulberry cultivars and were subjected to analysis of variance (ANOVA) and regression analysis to study the relative importance of cultivars and seasons on the disease incidence and the cultivars response.

Leaf yield loss in relation to PDI : Leaf yield loss was estimated by weight from the healthy and diseased plots by following the paired plot technique (Le Clerge, 1971). One of the plots in each pair was protected by spraying recommended fungicide Chlorothalonil (0.2% conc.) after noticing initial appearance of leaf rust symptoms. Un-protected plot kept as it was for the disease development. Leaf yield was estimated at leaf harvest stage both from protected and un-protected plots by using the formula (Gandhi and Chand, 1985).

$$\text{Leaf yield loss (\%)} = \frac{\text{Leaf yield in Healthy plots} - \text{Leaf yield in diseased plots}}{\text{Leaf yield in healthy plots}} \times 100$$

ANOVA and regression analysis were worked out of study relationship between leaf yield loss and disease severity (Jain *et al.*, 1997).

RESULTS AND DISCUSSION

Seasonal influence on leaf rust severity (PDI) in different mulberry cultivars : The study revealed significant variations in the degree of disease severity among the test cultivars in different seasons. The disease incidence was more prevalent in winter season as compared to rainy season. Leaf rust disease symptoms were generally absent in all the cultivars during summer season in all the three years of study. The absence of disease in summer season in all the test cultivars could be attributed to scanty rainfall, dry spell over the whole crop period. In addition, the prevailing high temperature for the longer duration of the day might have a detrimental effect on the pathogen survival and intern on the disease onset and its progress. Further it is also reported that the photosensitivity of the mulberry plant exposed to longer growth period might have prevented the disease development due to the physiological imbalance and poor compatibility between the host and pathogen interaction. Similar results have been reported for different foliar diseases on mulberry and on other agricultural crops. The leaf spot disease of mulberry occurred after the on set of monsoon and disappeared when the crop is in summer season (Siddaramaiah and Hedge, 1990). Gunasekhar *et al.*, 1994, Srikantaswamy *et al.*, 1997 reported varying degrees of leaf spot and leaf diseases in six promising mulberry cultivars. The degree of disease incidence like leaf spot and leaf rust and powdery mildew was negligible in summer season.

During rainy season, maximum disease index was recorded in S-13 (22.50%) > local (14.3%) > K-2 (12.56%) > S-36 (7.96%) and it was minimum in MR-2 (2.0%) followed by S-34 (3.1%) and S-54 (4.1%) in the year 2006. During the year 2007, highest disease index was recored in K-2 (29.16%) > S-36 (12.6%) > Local (9.6%). Cultivars S-34 (3.68%) > MR-2 (2.96%) > S-54 (2.6%) showed least disease index. Similar trend was observed in K-2 (26.4%) and in S-13 (24.06%). The test cultivars Local, S-34, S-36, S-54 and MR-2 showed poor disease index during the year 2008 (Fig - 1). Three years pooled data also had shown the highest disease index in S-13, S-36 and K-2 (Table - 1). During winter season, maximum disease index was recored S-36 (23.0%) > S-13 (22.1%) and K-2 (10.0%). Least disease index was recorded in other test cultivars Local, S-34, S-54 and MR-2 in the year 2006. Similar trend was observed in the subsequent year 2007 and 2008 (Fig. - 1). Three years pooled data also had shown the highest disease index in S-13, S-36 and K-2 (Table - 1). Incidence of leaf rust in rain season could be due to intermittent rainfall and higher relative humidity influencing the spores dispersal, initiation of disease symptoms and its progress. High leaf rust disease prevalence in winter season could be attributed to high relative humidity, presence of fog/dew for more than six hours in the atmosphere during night/early morning hours resulting in formation of leaf wetness followed by dry weather during day time influencing the disease development. The splashing rain dispersed the pathogenic propagules in early crop period and subsequent fog and dew becoming predisposal factors for these spores to germinate and increase in numbers of rust infection in winter season. Gunasekhar *et al.*, 1994 and Srikantaswamy *et al.*, 2000, 2006 have reported that, the leaf

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rust disease incidence was generally high in winter followed by rainy season in three southern mulberry growing states (Andhra Pradesh, Tamil Nadu and Karnataka). Gulya *et al.*, 1991 found that the presence of dew in atmosphere creates an ideal condition for the rust infection in sunflower with increasing dew period. Further, in several plant pathogen interactions, the intensity and atmospheric precipitation have been reported to play a vital role in the disease onset and progress (Shaw *et al.*, 1990; Awasthi and Kolte; 1994). Thus the results are in accordance with earlier workers. The high disease severity in S-36, K-2, S-13 and local indicated the degree of susceptibility for the leaf rust disease. The cultivars S-34, S-54 and MR-2 showed low percentage of disease and this could be attributed to the tolerance capacity of the cultivars.

Leaf yield loss (%) in different cultivars : During rainy season, significant variations were noticed with respect to leaf yield in different cultivars in different seasons. Highest leaf yield loss was recorded in S-13 (14.5%) > K-2 (12.5%) and Local (8.1%). Minimum leaf yield was recorded in other test cultivars like S-34, S-36, S-54 and the loss was nil in MR-2 during the year 2006. Similar trend was recorded in the subsequent years 2007 and 2008 (Fig - 2). During winter season, highest leaf yield loss was recorded in S-36 (19.0%, 31.1% and 18.6%)> S-13 (19.3%, 12.3% and 15.1%)> K2 (6.7%, 17.5% and 23.5%). Least leaf yield loss was recorded in S-34, S-54, Local and loss was nil in MR2 in all three years of study 2006, 2007 and 2008 (Table - 1 and Fig - 2). The cultivar S-36 was greatly influenced for the higher degree of disease severity and yield loss during the winter season when compared to the rainy season.

Relationship between the disease severity and yield loss was best described by a linear regression model with significant regression co-efficient in all the test cultivars. The leaf yield loss was high in S-36 (0-.92%), K-2 (0.90%), S-13 (0.81%) and local (0.69%) for every unit increase in PDI by showing high co-efficient determination more than 80% (r^2), where as in other test cultivars S-34, S-54, and MR-2 the r^2 values are very less. The leaf yield loss was positively and significantly associated with percentage of disease index (Fig - 3). The pooled data irrespective of the cultivars were also shown a definite linearity between PDI and yield loss in both rainy and winter seasons of the respective years and also for pool of three years by showing high co-efficient determination more than 90% (Table - 2). However the loss was found high in winter season for every unit increase in PDI. The regression yield loss model derived for rainy and winter season are Y = -2.95 + 0.85X** R2 = 0.95 and Y = - 2.73 + 0.92X** R2 = 0.98 respectively. The present investigation in clearly indicating the significant influence of winter season for disease initiation, progress and leaf yield loss in different mulberry cultivars. Burleigh *et al.*, (1972) have reported the clear cut linearity between the leaf rust and wheat yield, yield

Table 1 : Leaf yield loss and PDI in different mulberry cultivars (Pool of three years).

Cultivar	Rainy season		Winter Season	
	PDI	Yield loss (%)	PDI	Yield loss (%)
Local	9.40 ± 2.89	4.53 ± 1.97	7.75 ± 1.23	4.83 ± 1.51
K - 2	22.71 ± 5.16	17.04 ± 5.30	20.40 ± 5.81	15.90 ± 4.91
S - 13	22.90 ± 0.59	17.87 ± 1.88	20.43 ± 2.19	15.57 ± 2.03
S - 34	3.56 ± 0.24	0.97 ± 0.07	5.00 ± 0.06	0.93 ± 0.06
S - 36	8.02 ± 2.63	1.44 ± 0.48	29.07 ± 5.53	22.9 ± 4.10
S - 54	3.07 ± 0.52	0.24 ± 0.24	3.28 ± 0.86	0.00
MR - 2	2.69 ± 0.35	0.00	3.19 ± 0.88	0.00
SE	2.54	2.30	3.17	2.65
CD (5%)	7.85	7.09	9.78	8.17

Table 2 : Relationship between leaf rust severity and leaf yield loss.

Sl. No.	Year	Season	Regression equation	R^2
1.	2006	Rainy	Y = - 1.89 + 0.65 X**	0.99
2.	2007	-do-	Y = - 3.98 + 0.99 X**	0.98
3.	2008	-do-	Y = - 2.30 + 0.82 X**	0.96
4.	2006	Winter	Y = - 2.81 + 0.92 X**	0.96
5.	2007	-do-	Y = - 1.85 + 0.82 X**	0.99
6.	2008	-do-	Y = - 4.57 + 1.07 X**	0.98
Pool of three years				
1.	Rainy		Y = - 2.95 + 0.85 X**	0.95
2.	Winter		Y = - 2.73 + 0.92 X**	0.92

** Significant at p<0.01

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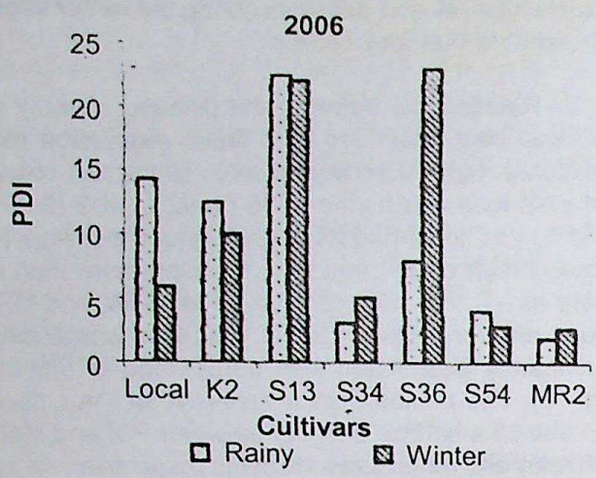
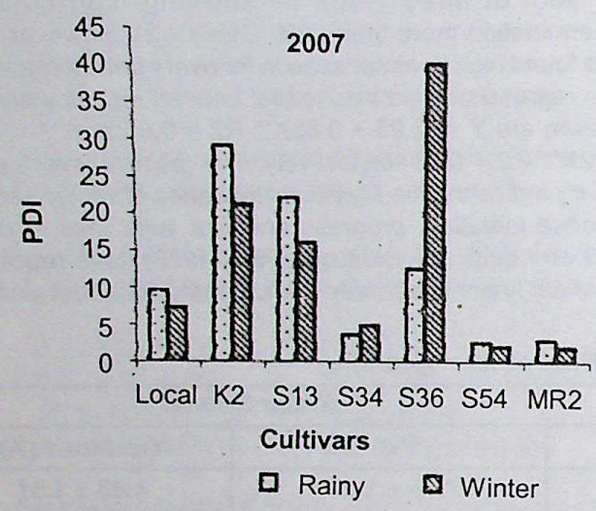
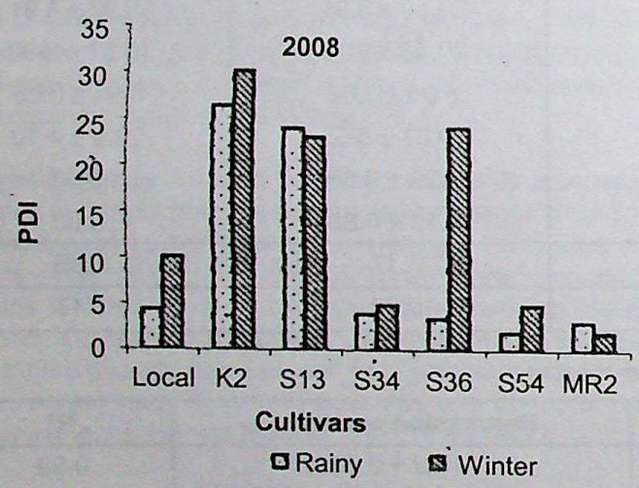
Table 1 : Leaf rust percentage disease index in different cultivars.

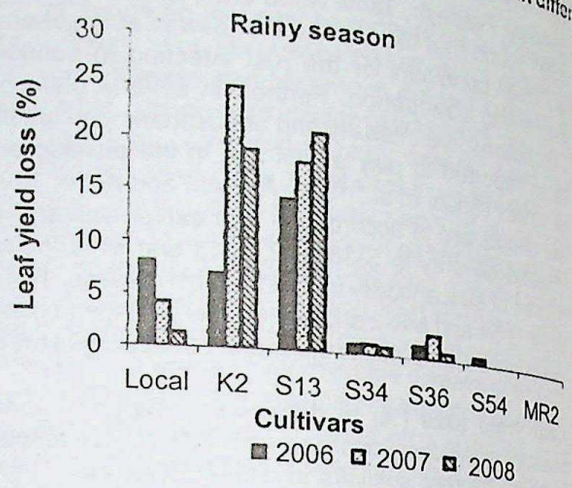


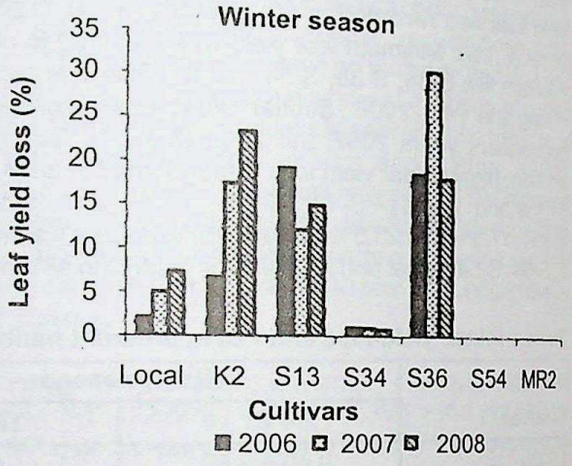




loss increased significantly with increasing the rust severity. In the present study, the derived regression equation also had shown clear cut linearity beween PDI and yield loss in both rainy and winter seasons of the respective years and pool of three years data (Table - 2). Varying degree of leaf yield loss in different cultivars in different seasons presumably due to cultivar resistance characteristics. Similar observations have been reported in leaf spot and powdery mildew diseases in mulberry. Srikantaswamy *et al.,* (1997, 2000) have reported the effect of different disease severity level of leaf spot and

Table 2 : Leaf yield loss due to leaf rust disease in different cultivars.





powdery mildew on leaf yield loss in K-2 cultivar and observed a direct linearity between disease severity and leaf yield loss in rainy and winter seasons. Based on the present investigation we can infer that among the three seasons rainy/winter seasons has profound influence and facilitates the occurrence and severity of leaf rust. Cultivars MR-2, S-34 and S-54 can be considered as "slow rusters" and becoming important sources for further breeding programme to increase the level rust resistance.

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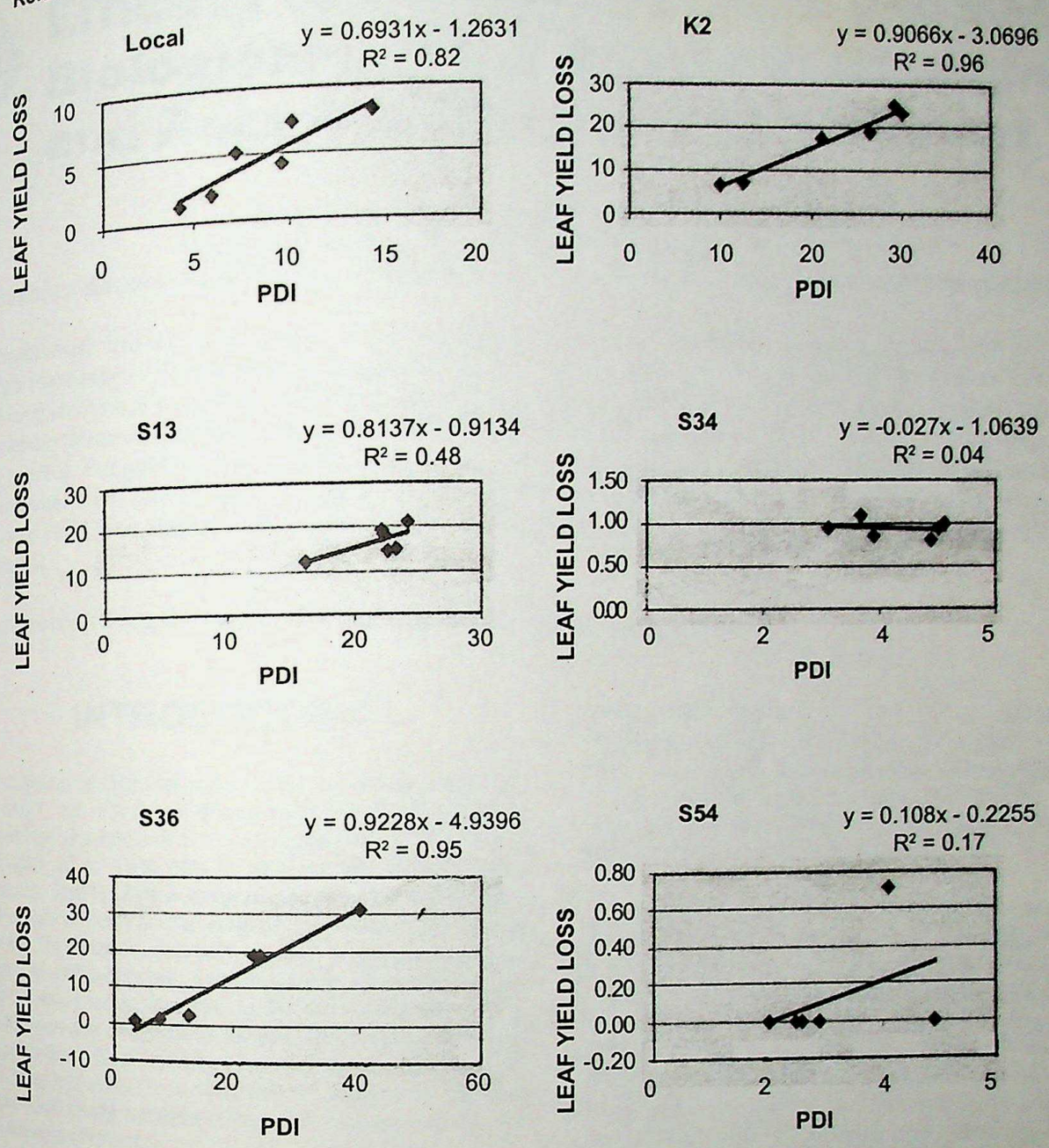
Fig 3 : **Relationship between leaf rust disease severity and leaf yield loss in different mulberry cultivars.**



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Efficacy of Mixture of Fungal and Bacterial Biological Control Agents in Managing Wilt and Root Rot Diseases of Safflower

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Greenhouse and field experiments were conducted to evaluate the efficacy of 6 fungal biogents in combination with *Pseudomonas fluorescens* in managing wilt and root rot of safflower. The biogent formulations were applied as seed treatment @ 4g/Kg of seed (2g fungal biogent + 2g *P. fluorescens*). In green house experiments seedling mortality was more in Nira cultivar than PBNS-43. In general biogents were able to control mortality as compared to control. Fungal biogents *viz., T. varide, T. harzianum* and *Gliocladium virens* (*T. virens*) in combination with *P. fluorescens* were found more effective than other biogents tested in green house and field experiments also. Grain yield was significantly higher in all the biogents expect *A. fumigatus* + *P. fluorescens* as compared to control.

Keywords : Fungal biogents, *P. fluorescens*, Wilt and Root rot, Grain yield.

INTRODUCTION

Safflower suffers from mortality due to wilt (*Fusarium oxysporum* f. sp. *carthami*) and root rot (*Macrophomina phasolina*) heavily. Loss of the crop to the tune of 61% due to wilt and upto 10 to 15% due to root rot has been reported (Anonymous, 2001). Since these diseases are soil borne in nature it is very difficult and uneconomical to manage them by chemicals. Resistant varieties having good agronomical attributes are not available. In this situation biogents having high rhizosphere competence, antibiotic producing ability, ecofriendlyness are of prominence in managing the soil borne disease problems. Considering these attributes combinations of fungal biogents with *Pseudomonas fluorescens* have been tried in present study for their efficacy in management of wilt and root rot of safflower.

MATERIAL AND METHODS

There experiments (two greenhouse and one field experiment) were conducted to find out the efficacy of combination of fungal (*Trichoderma viride, T. harzianum, G. virens, Aspergillus nigar, A. fumigates, Paecilomyces varioti*) with bacterial (*Pseudomonas fluorescens*) formulations in managing wilt and root rot of safflower.

In greenhouse experiments, in one of the set, field soil naturally infested with *F. oxysporum* f. sp. *carthami* and *M. phaseolina* was used in pots while in another set of experiment this soil was mixed with cultures of aforesaid organisms in combination to make it artificially sick. Soil was inoculated by mixing growth of these organisms at the rate of 200g of growth separately in 900 cc soil - compost - sand mixture. Fungal biogents were mass multiplied on sterilized potato dextrose broth for a week's period and the homogenized growth was mixed in sterilized carrier (lignite) in 1 : 3 (V/W) proportion as per procedure described by Chavan (1998). The product had a population of 2 x 10^6 CFU/g.

P. fluorescens was multiplied on King's B broth upto 72 hrs on a mechanical shaker and formulation was prepared by mixing 400 ml of bacterial growth containing 9 x 10^8 CFU/ml in one kilogram of sterilized lignite. Formulations of fungal biogents and *P. fluorescens* were used to treat the seeds of safflower cultivars *i.e.,* Nira and PBNS 43, susceptible and moderately susceptible to wilt, respectively @ 4g/Kg of seed (2g fungal antagonist + 2g *P. fluorescens*) using gum arabic as stricking agent. The treated seeds were air dried in shade and used for sowing @ 5 seeds / pot. There were 14 treatments including untreated control, replicated thrice in each set of experiment

For field experiment a plot naturally infected with wilt and root rot diseases upto 10-15% in previous years was selected. Aforesaid formulations of biogents used to treat the seed of two safflower cultivars as mentioned earlier. Inoculation of *F. oxysporum* f. sp. *carthami* and *M. phaseolina* was separately multiplied on sterilized corn meal sand medium for a weeks period in ploypropylene bags. Five kilograms of inoculum in polypropylene bage of each pathogen was incorporated separately in experimental plots below the rows where seeds are to be sown. Experiment was laid out in factorial randomized block design with three replications and 14. treatments including untreated control. Normal agronomic practices were followed for raising the crop. Field was irrigated

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Table 1 : Mortality (%) of safflower as influenced by seed treatment with biogents.

Sr. No.	Treatments	Pot Study Mean mortality (%) at 50 DAS		Mean mortality at 90 DAs	Field study		
		Field soil	Artificially made sick soil		Per cent decrease over control	Grain yield (Kg/ha)	Per cent increase over control
A.	**Cultivars (C)**						
	1. Nira	19.52 (25.55)	57.17 (51.46)	14.94 (22.34)	--	981	--
	2. PBNS – 43	20.48 (26.55)	43.80 (42.18)	12.67 (20.59)	15.29	1050	7.03
	S.E. +	0.88	1.09	0.40	--	--	--
	C.D. at 5%	N.S.	3.15	1.18			
B.	**Biogents**						
	1. *T. viride* + *P. fluorescens*	11.67 (19.79)	36.67 (37.22)	8.50 (16.87)	60.08	1288	78.64
	2. *T.harzianum* + *P. fluorescens*	11.67 (19.79)	28.33 (32.00)	8.45 (16.92)	60.13	1164	61.44
	3. *G. virens* + *P. fluorescens*	13.33 (21.14)	31.67 (34.10)	9.80 (18.22)	53.97	1100	52.57
	4. *A. nigar* + *P. fluorescens*	20.00 (26.31)	60.00 (50.99)	15.48 (23.03)	27.29	956	32.59
	5. *A. fumigatus* + *P. fluorescens*	23.33 (28.78)	48.33 (43.99)	18.84 (25.68)	11.50	878	21.78
	6. *P. varioti* + *P. fluorescens*	25.00 (29.64)	53.33 (46.96)	14.29 (22.15)	32.88	997	38.28
	7. Untreated control	36.67 (37.22)	95.00 (82.45)	21.29 (27.40)	--	721	--
	S.E. +	1.65	2.04	0.76	--	80	--
	C.D. at 5%	4.78	5.89	2.21	--	234	--
C.	**Interaction (C x B)**						
	S.E. +	2.33	2.88		--	114	--
	C.D. at 5%	N.S.	N.S.		N.S.	N.S.	--

Figures in parentheses are arcsine values and statistics was applied to them.

4 times including pre sowing irrigation. Observations for disease incidence (% mortality) were recorded at 30, 45, 60, 75 and 90 days after sowing.

RESULTS AND DISCUSSION

In pot experiments, mortality of safflower did not differ significantly between cultivars in fields soil but in artificially inoculated soil, seedling mortality was significantly higher in cultivar Nira (susceptible) than PBNS-43. This difference in mortality can be attributed to difference in susceptibility of the cultivars. In general both the species of *Trichoderma* (*T. harzianum* and *T.viride*) and *G.virens* in combination with *P. fluorescens* were found more effective than other biogents tested in this study (Table 1).

In field experiment, Nira was found more susceptible than PBNS 43 for mortality of safflower. However, grain yield was not significantly influenced between safflower cultivars. In general biogents significantly reduced safflower mortality was observed as compared to control. Minimum safflower mortality was observed in both the species of *Trichoderma* and *G. virens* combined with *P. fluorescens* which was significantly less than rest of the treatments. Other promising biogents include *P. varioti* and *A. fumigatus* combined with *P. fluorescens*. Biogents reduced safflower mortality from 11.5 to 60.1 per cent at 90 days after sowing. Grain yield was significantly higher in all the biogents except *A. fumigatus* + *P. fluorescens* combination as compared to control (Table 2).

Usefulness of *T. viride* + *P. fluorescens* in reducing wilt and root rot of different crops has also been reported by various workers (Gholve and Kurundkar, 2002; Behere *et al.*, 2002; Prased, 2003 and Sajeena *et al.*, 2003). These observations support finding of present study. As regards to *T. harzianum* + *P. fluorescens*, Singh (2000) reported effectiveness of *T. harzianum* in managing wilt induced by *Fusarium udum*. Also Behere *et al.*, (2002) reported effectiveness of this fungus in managing root rot caused by *Macrophomina phaseohina*. These observations are similar to the finding of present study. Usefulness of *G. virens* in managing seedling mortality due to wilt and root rot diseases in present study is on similar line with those reported by Tewari and Mukhopadhyay (2001) and Behere *et al.* (2002).

Increase in grain yield due to biogents *T. viride*, *T. harzianum* and *G. virens* has been reported by Sajeena *et al.*, (2003) is agreeing with the present finding.

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Biosorption of Heavy Metals from Industrial Waste Water by Brown Alga *Sargassum ilicifolium* (Turner) C. Agardh

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Biosorption is emerging as a cost effective and highly efficient method for the removal of heavy metals from industrial waste water. Ability of a brown alga *Sargassum ilicifolium* to remove heavy metals from industrial waste water was assessed. Batch experiments were conducted to determine adsorption properties of the *Sargassum ilicifolium* and it was observed that the biosorption capacity was strongly pH dependent. Results demonstrated that about 90% removal of lead by the dried algal biomass from polluted water was within 30 minutes. The order of efficiency in the process of adsorption of heavy metal was Pb > Cu > > Ni > Cr. The biosorption mechanism involved mainly ion exchange reactions between the heavy metal ions present on the algal surface as confirmed by SEM-EDAX. The Chemical nature of metal-algal interaction has been Elucidated employing XRD Analysis.

Keywords : Biosorption, Heavy metals, SEM-EDAX, Waste water, XRD.

INTRODUCTION

Disposal of Heavy metals into water bodies causes a major concern because of their non-biodegradability and toxic nature as well as tendency for accumulation throughout food chain (An, *et al.*, 2001).Industrial wastes contain harmful metal ions which affect all the biota and may further cause nuisance to humans (Rahman *et al.*, 2004). Existing technologies for metal ion removal from waste water are costly and include use of ion-exchange resin, solvent extraction, electrolytic and precipitation process, Electro dialysis and Membrane technology (Strick and Staden, 2000). Most of these methods are neither effective nor economical. Natural adsorbents have been proposed for removing metal ions due to their better adsorption capacity (Brauner and Wood 2002). Recently biosorption has emerged as an attractive alternative to combat heavy metal contamination because of its good selectivity, high efficiency, low cost, broad applicability and strong ability of precious metal ion recovery (Kiran *et al.*, 2007, Yin *et al.*, 2008).

Microorganisms such as algae, yeast, bacteria and fungi have been widely evaluated as biosorbents for removing metal ions from aqueous solutions (Razmovski and Sciban, 2008, Gupta and Rastogi, 2008). Dead algal biomass has been used for metal ions adsorption due to the characteristics of the functional groups present in these materials. Present study investigates the application of a macro algal material in the removal of toxic heavy metals like lead, copper, chromium and nickel present in the waste water. The process of biosorption is influenced by different parameters such as initial concentration of the metal, dose of the adsorbent and the contact time which are also studied. SEM-EDX and XRD analysis of the bisorbent was carried out to elucidate the biosorption mechanism.

MATERIAL AND METHODS

Fresh and mature thalli of *S. ilicifolium* were collected from Malvan and Kunakeshwar (District -Sindhudurg) located at latitude 16° 4'59"N (16.083°) and longitude 73°30'E (73.500°) along the west coast of Maharashtra. The algal material was cleaned, washed, sundried and then milled and stored at room temperature in airtight containers.

Metal biosorption : Before the experiment, the initial concentrations of heavy metals in the waste water were determined on AAS. An accurately weighed 100 mg biomass was added in a flask containing 50 ml waste water and it was agitated on a rotary shaker at constant speed (170 rpm) for 30 min. Then the biomass was removed by filtrations and the waste water was analyzed on AAS.

The metal uptake, Q, was determined by using the following equation.

$$Q = V (C_I - C_F) / M$$

Where 'Q' represents the amount of metal taken per unit mass of the biomass (mg/mol g^{-1}), V is the volume of the solution (L), M the dry mass of the algae (g), C_I and C_F are the initial and final concentrations (mol m^{-3}) respectively.

SEM - EDX and XRD Analysis : Surface morphology of the biosorbent was studied using the original powdered samples under the scanning electron microscope (Jeol- Jsm 6360). EDX

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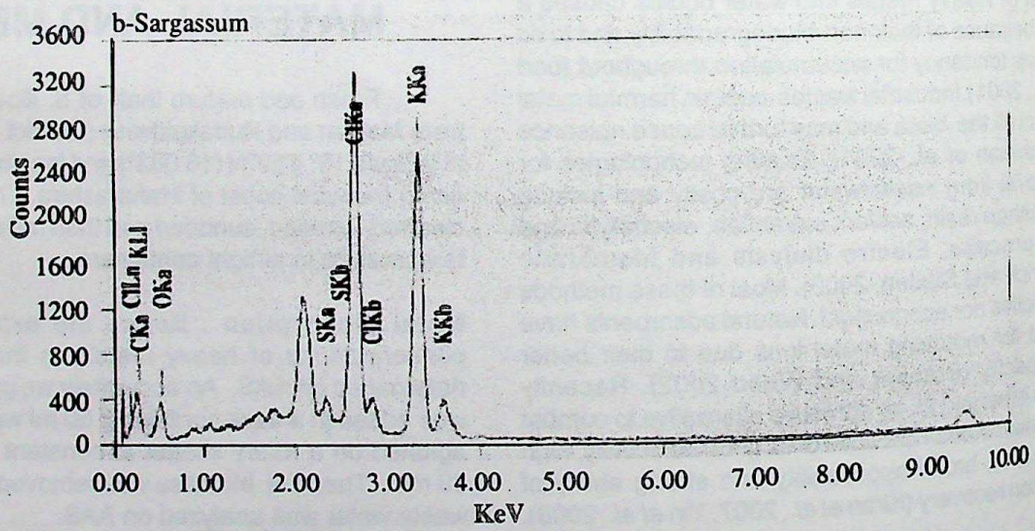
Table 1 : Uptake of Heavy metals from Industrial waste water by *Sargassum ilicifolium* (Original pH-2.87)

Metal	Initial concentration of Heavy Metal in waste water (ppm)	Final concentration of Heavy Metal in waste water(ppm)	Q max	Mmolg^{-1}	%
Copper	1.150	0.405	372.5	5.146	64.78
Lead	2.007	0.242	893.5	4.309	89.03
Chromium	0.261	0.212	29.5	0.567	22.61
Nickel	0.121	0.091	15.0	0.255	24.79

Fig.1 SEM and EDX micrograph of *Sargassum ilicifolium* biomass magnification X750



was carried out in order to characterize the metal distribution on the surface of the algal biomass. The crystalline phases in the *S. ilicifolium* were characterized with an X-ray diffractometer. (XRD) at a scan speed of 1.2 min^{-1}.

RESULTS AND DISCUSSION

Removal of heavy metals from waste water was detected to its original pH.The maximum removal of copper was observed while chromium was less removed. The uptake of heavy metals was in order Pb > Cu > Ni > Cr. The biosorption percentage of lead was highest due to the acidic pH.

Characterization of biosorbent : The SEM analysis of *S. ilicifolium* (Fig.1) revealed important information on surface morphology. In these micrographs structures with large surface area were evident. EDX (X-ray microanalysis) can provide the information regarding the elemental characteristic of the biomass. The EDX spectrum of the biosorbent exhibited distinct peaks of carbon, oxygen, potassium, sulphur, magnesium indicating substantial presence of the elements in the biomass.

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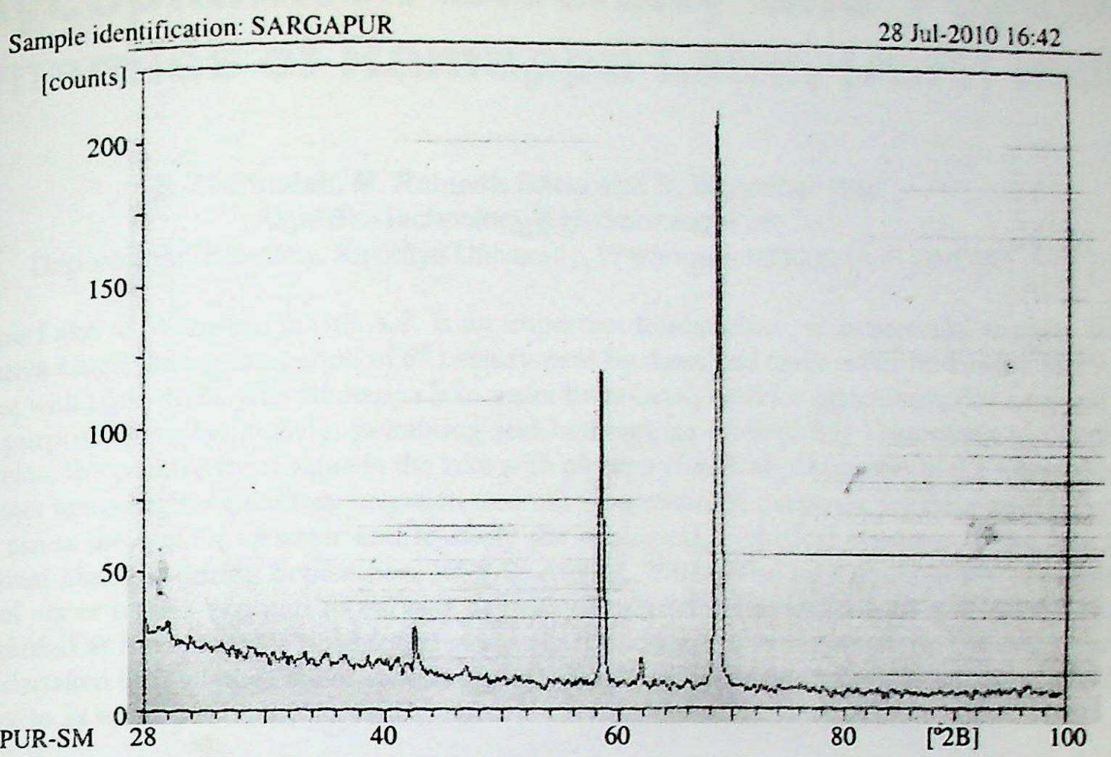
Fig.2 XRD pattern of *Sargassum ilicifolium* biomass.



Ion (K, Mg, Na, Al, Si, S, Cl, Zn) release from the biomass is a frequently observed event that has significance in deciphering the mechanism of the metal biosorption. It has been suggested that ion -exchange rather than sorption to free sites could be a relevant mechanism for the binding of metal ions onto the biomass (Lopez *et al.*, 2000). Several previous reports have demonstrated extensive K release from biomass following metal uptake, suggesting K release to maintain ionic balance across the membrane. In present case, the overall observation strongly indicates the possibility of binding with the biomass by displacing the cellular potassium through an ion-exchange mechanism.

X-ray powder diffraction analysis : The XRD pattern showed distinct peaks at different positions. (2θ=68.750°, 61.830°, 58.570° and 42.630°). Based on the d-spacing values (2.0277, 2.2283, 2.3405 and 3.1496 A) these peaks are attributable to the presence of crystalline structure on the biomass. The crystalline formation following long period of metal accumulation could be due to the complexation of metal nucleation and metal precipitation in crystalline state (Lopez *et al.*, 2000).

The SEM-EDX Analysis indicated the capacity of *Sargassum ilicifolium* in the biosorption of metal ions. The maximum removal of Pb indicated that a highly acidic pH is required where as for Chromium and Nickel removal this pH may not be suitable and this strongly recommends a moderate pH for the process of metal biosorption.

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Phycoplankton Diversity and Population Dynamics of Ramappa Lake, (A.P.) India

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The Ramappa Lake of Warangal (Dist.) A.P. is an important tourist place, with beautiful temples built by mighty Kakatiya kings, during the period of 8th century near by them and these water bodies are surrounded by thick forest with rocky hills. The Ramappa lake water body being used for agriculture, drinking and other recreational purposes (boating, fishing, swimming and bathing), an attempt has been made in the present study to correlate the periodicity of algae in the lake with physico-chemical parameters of the water. Further, these lake water are using for drinking, irrigation and other recreational purposes, we have approached the problem, to assess the quality of water and to study the ecological, biological concerns of the lake under various seasonal changes during September, 1999 to August, 2001. The lake under study are important component of water bodies exposed to varying degrees of cultural eutrophication to some extent and are therefore regarded as more suitable production studies in the scheme of its reclamation. Hence, the present study was undertaken to elucidate certain aspects of phytoplanktons of selected lake of Warangal district (A.P.) with reference to its water pollution.

INTRODUCTION

Phytoplankton forms the vital source of energy as primary producers and serves as a direct source of food to the other aquatic plants and animals. It accounts substantially for the organic production of waters ways. They provide information on the productivity of the environment. In India some lakes and reservoirs have been studied for the water quality and fisheries (Nautiya *et al.* 1988; Kartha and Rao, 1992, Pandey, 1993; Ravikumar *et al.* 2006; and Tas and Gonulal, 2007 and Sukumaran and Das,1999). There are many such aquatic ecosystem that remain unexplored. The Ramappa lake has also not received due attention. Therefore, the present study was made to know the influence of physico-chemical parameters of water on phytoplankton population and their seasonal changes in the lake under study.

MATERIAL AND METHODS

The water samples collected from the lake in bottles were brought to the laboratory for analysis as per the standard methods described by APHA (1985) and Trivedy and Goel (1986). Phytoplanktons were identified by using standard key books (Patrick 1943, Suxena and Venkateshwarlu, 1968, Philipose, 1967, Desikachary, 1959, Prescott, 1951 and Smith, 1950.

RESULTS AND DISCUSSION

The water temperature varied from 24°C to 31°C. The turbidity of water fluctuated from 0.01 to 6.00 NTU, conductivity annual average value varied from 0.68 to 0.86 m mhos/cm in 1999-2000 and from 0.56 to 7.8 m mhos/cm in 2000-2001. The Transparency of water varied from 24 to 125 cm, TDS ranged from 140.00 to 680.00 mg/l, TSS fluctuated from 170.50 to 480.20 mg/l respectively. The bottom substrata of the lake appeared to be sandy loamy in nature.

The pH values were ranged from 7.1 to 8.8 during the period of study and Dissoved Oxygen was varied from 6.6 to 11.9 mg/l. In Primary productivity the GPP values ranged from 0.1168 to 2.67613 mg c/hr/l, NPP values varied from 0000 to 2.7454 mg c/hr/l and $Resp^N$ values varied from 0.1134 to 1.9542 mg c/hr/l. The oxidation-reduction potential fluctuated between 0.184 to 0.420 and while oxidation-reduction index values ranged from 24.67 to 28.69 respectively. The Biochemical oxygen demand varied from 6.2 to 21.4 mg/l, while Chemical oxygen demand values ranged from 0.31 to 1.1 mg/l. Silicates, Free CO_2, Phosphates, Total Hardness and Chloride values varied from 0.0001 to 0.003 mg/l; 2.2 to 43.20 mg/l; 0.05 to 0.84 mg/l; 100 to 300 mg/l; and 11.8 to 134.1 mg/l respectively. From the total alkalinity the OH alkalinity was observed only in three months during the two years of study. Its values ranged from 30 to 100 mg/l and values of PA varied from 20-240 mg/l. The total alkalinity *i.e.*, amount of bicarbonates varied from 20 to 300 mg/l and they could not find nearly in six months. The nitrate, nitrite and ammonia contents were not observed during the study period. Throughout the period of study the contents of Chromium, Cadmium, Cobalt, Lead, Mercury and Zinc were not detected.

The phytoplankton members comprised of 60 genera of which 18 belonged to Bacillariophyceae (14.35%), 33 to Chlorophyceae (12.50%), 26 to Cyanophyceae (72.25%) and

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5 to Euglenophyceae (0.90%). Highest number of individual was represented by Cyanobacteria (7620590), followed by Diatoms (718260), Green algae (451353) and Euglenophyceae (82935). A total of 82 forms of phytoplanktons were recorded. The total percentage of phytoplanktons were 90.75% and other are 9.25%. The seasonal abundance of the prominent phytoplanktons of Ramappa lake have shown two diatoms, *Navicula exigna* and *Pinnularia* sp., two blue greens, *Merismopedia glauca, Lyngbya ceylanica* and a member of green algae, *Pediastrum duplex*. The Nygaard compound index shown the range of variation from 1.9 to 6.6 with the average value of 4.16 during the study period.

The phytoplankton population was stable during the months of April and May. The density slowly declined during the June and Pre-monsoon period and lowest value was during the month of December 2000. In the present study, the maximum phytoplankton production coincided with the optimum water depth of one meter. This is an agreement with the Das *et al* (2003) in some fresh water bodies. In the present investigation it may be noted that the phytoplankton population of the lake seems to be closely related with the seasonal variations in the study.

Table 1 : Observed phytoplankton species in Ramappa lake.

S. No.	BACILLARIOPHYCEAE
1.	*Acananthes* sp
2.	*Amphora* sp
3.	*Amphipleura pellucida*
4.	*Cymbella sturbergii*
5.	*C. hustedii*
6.	*Diatoma* sp
7.	*Dioponeis* sp
8.	*Fragilaria* sp
9.	*Fragilaria crotononensis*
10.	*Frustulia* sp
11.	*Gomphonema* sp
12.	*Mastoglosia* sp
13.	*Navicula exigna*
14.	*N. ryptocephala*
15.	*Nitzchia* sp
16.	*Pinnularia* sp
17.	*Synedra* sp
18.	*Tabellaria* sp
	EUGLENOPHYCEAE
19.	*Euglena acus*
20.	*E. viridis*
21.	*E. caudata*
22.	*Trachelomonas* sp
23.	*T. curta*
	CYANOPHYCEAE
24.	*Anabaena iyengarii*
25.	*A. variabilis*
26.	*A. vagincola*
27.	*Aphanocapsa montana*
28.	*A. litorales*
29.	*Aphanothece pallida*
30.	*Aphanozomenon* sp.
31.	*Arthrospira* sp
32.	*Gloeocapsa atrata*
33.	*Gloeotrichia natans*
34.	*Gloeotrichia pisam*
35.	*Merismopedia glauca*
36.	*Microcystis aeruginosa*
37.	*M. flos - aquae*
38.	*Nostoc linchia*
39.	*N. sphaerium*
40.	*Oscillatoria formosa*
41.	*O. tenuis*

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	O. limosa
42.	*Rivularia* sp.
43.	*Phormidium molle*
44.	*Spirulina laxissima*
45.	*Synechococcus* sp.
46.	*Trichodesmium* sp
47.	*Lyngbya ceylanica*
48.	*L. majuscule*
49.	**CHLOROPHYCEAE**
	Ankistrodesmus falcatus
50.	*Bulbochaete* sp.
51.	*Chara vulgaris*
52.	*Chlorella vulgaris*
53.	*Closterium ehrenbergii*
54.	*Cladophora glomarata*
55.	*Cl. crispatq*
56.	*Cosmarium botrytis*
57.	*C. leave*
58.	*C. phaseolus*
59.	*C. javanicum*
60.	*Euastrum verrucosum*
61.	*Hydrodictyon reticulatum*
62.	*Mougeotia* sp.
63.	*Nitella* sp
64.	*Oedogonium borisianum*
65.	*Oocystis gigas*
66.	*Pandorina* sp.
67.	*Pediastrum duplex*
68.	*P. ovatum*
69.	*Rhizoclonium hieroglyphicum*
70.	*Scenedesmus denticulatus*
71.	*S. dimorphus*
72.	*S. obliquus*
73.	*Sirogonium phacosporum*
74.	*Spirogyra acanthospora*
75.	*S. formosa*
76.	*Stigeoclonium* sp.
77.	*Tetraedron gracile*
78.	*T. quadratum*
79.	*Ulothrix* sp.
80.	*Zygnema czurde*
81.	*Zygnemopsis* sp.
82.	

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Studies on Seed Storage and Viability of some Forest Seeds of Family Leguminosae Found in Western Ghats

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Seeds of five species of leguminosae namely *Dalbergia melanoxylon* Guill & Purr *Dalbergia latifolia* (Roxb.), *Acacia catechu* Willd., *Acacia nilotica* (Linn) Del., and *Acacia chundra* (Rottl,) willd. were stored for 24 months, the viability of the seeds was tested by 1.0% aqueous solution of 2,3,5 triphenyl tetra-zolium chloride every month. Six packaging materials namely open mouth bottle in refrigerator, open mouth bottle at room temperature, paper bag, polythene bag, desiccator at room temp. and desiccator in refrigerator were used. The seed took variable time for the complete loss of viability even in one kind of packaging material. Seeds stored in desiccators kept in refrigerator were considered as most superior then others.

Keywords : *Dalbergia melanoxylon, D.latifolia, Acacia catechu, A. nilotica, A. chundra,* Storage, Viability, Seeds.

INTRODUCTION

Leguminosae seeds are characterized by a prolonged viability and pronounced dormancy that persist for a long periods in soil. The purpose of storing the seeds of economic plants is to pressure the planting stocks from one season to another. As agriculture and forestry developed, man expanded his knowledge regarding both the requirements of seed for maintenance of viability and method of providing suitable storage condition. The informations on seed storage of forest trees, fruits trees and woody leguminous forest, species are scanty. Therefore, experiments were conducted to find out the safe storage and viability method for some of the species in leguminosae.

MATERIAL AND METHODS

Mature seeds of five different plants were collected and stored on different dates (Table 1) and in different ways. One lot was kept in plastic tubes of about 2" wide mouth covered and tied by two folds of cheese cloth. One lot of such plastic tube was stored in a refrigerator and the other at room temp. Another set of seeds were kept in brown paper bags. The top of the bag folded thrice and clipped. One bag was stored at room temp. and another bag kept in desiccator at room temp. and in desiccator in a refrigerator. One lot of seeds was kept in a small polythene bag of 250 gauge thickness with the mouth tied with a thread and stored at room temp.

Seeds stored at room temp. were kept in the drawer of wooden herbarium cabinet at room temp. The approximate temp. of refrigerator was 5 - 7°C. The relative humidity of desiccator was about 65% average mean monthly temp. and relatively humidity varied between 12°C and 33°C, the correct temp. occurred during December to February and highest during May to July, average monthly relative humidity ranged between 61 to 91 per cent, the lowest values always occurred between April to June and highest between August to October.

The seed moisture was determined in temp. controlled electric oven. One hundred seeds of each kind were weighted in duplicate and dried in an oven at 103 ± 1°C for 24 Hrs. Seeds were removed from oven and transferred to desiccator. They were weighted again and the moisture percentage was expressed on wet weight basis (Table 1).

Viability of seeds was tested 1% aqueous solution of 2, 3, 5 triphenyl tetrazohum chloride. The test was conducted every month. Seeds were reandomly selected and soaked for 17 hours in water. After removal of seed coat the seeds were rinsed with water, soaked in tetra zolium chloride solution and kept in darkness for 6 - 8 hrs. Seeds were then rinsed twice and kept in water to avoid drying and were then evaluated for viability.

RESULTS AND DISCUSSION

1. Storage at room temperature : The seeds were kept in open mouth bottle covered with cheese cloth. The initiation of loss in viability was obsorbed after one month of storage in *Dalbergia melanoxylon* Guill & Purr *Dalbergia latifolia* Roxb., *Acacia catechu* Willd., *Acacia nilotica* (Linn) Del., and *Acacia chundra* (Rottl,) willd. *Acacia chundra* (Rottl.) willd and after four months in *Acacia catechu* Willd. and after seven month *Acacia nilotica* (Linn) Del. and *Acacia chundra* (Roffl) Willd. after 5 month and *Dalbergia melanoxylon* Guill & Purr *Dalbergia latifolia* after 24 month of storage. In *Acacia* species 90% seeds loss their viability while in *Dalbergia* species 50 per cent seeds loss their viability.

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Table 1 : Details of different plant species and their seeds used in the experiments.

Sr.No.	Name of the Plant and species	Date of Collection	Date of Storage	Seed Moisture Content %
1.	*Dalbergia melanoxylon* Guill & Purr	30/11/09	08/12/09	6.97
2.	*Dalbergia latifolia* Roxb.	30/06/09	08/07/09	5.08
3.	*Acacia catechu* Willd.	08/05/09	13/05/09	6.95
4.	*Acacia nilotica* (Linn) Del.	20/05/09	27/05/09	0.67
5.	*Acacia chundra* (Rottl,) Willd.	15/05/09	15/05/09	29.09

2. Kept in paper bags: Initializations of loss of viability was observed only after two months in *Acacia* species and after 28 months in *Dalbergia* species.
3. Polythene Bags: The initiations and pattern of loss of viability was same as in case of brown paper bags. About 80-90% loss of viability was observed after 17 and 25 months of storage. Polythene bags considered suitable material as compared to open mouth bottle because they show better storability.
4. Kept in Desiccator: Initiations and pattern of loss of viability was observed after 8 months in *Acacia* species and after 18 months storage in *Dalbergia* species.
5. Storage in Refrigerator : Open mouth bottle kept in refrigerator covered with cheese cloth, Viability of seed lost rapidly within 4 to 5 months of storage in *Acacia* species and eleven months after storage in *Dalbergia* species.
6. Kept in Desiccator: The initiations and pattern of loss of viability was observed in different *Acacia* species after four months of storage and large number of the seeds about 90% lost then viability after 30 months of storage. In *Dalbergia* species. The loss of viability observed after 7 months of storage and then may remain viable even after 36 months of storage in desiccator.

Seeds of five different forest plants of family leguminosae stored in refrigerator and kept in desiccator maintained viability for longer duration. It was apparently because of low relative humidity and temp. during storage seed kept in desiccator and stored in refrigerator and kept in desiccator because of the variable room temp. Conditions during summer month seed experienced high temp. as about 40°C. The adverse effect of high temp. has been very well shown and documented (Barton; 1935, 43.54, 61, Myres, 1946; Atten 1957; Bass *et al*, 1963; Scharpt 1970).

Seeds of different leguminous forest species stored in polythene bags could not maintain their viability for period as long as in case of desiccator which may be due to the fact that they were not sealed, therefore the seed moisture have fluctuated with the fluctuation of relative humidity of the atmosphere. High moisture coupled with high temp. were reported to bring down the viability rapidly (Whymper and Broadley, 1934; 1934b Moore, 1963; Bass *et al.* 1967; Ghosh and Nandi, 1981).

The seeds kept in open mouth bottle and stored at room temp. or in refrigerator and those kept in brown paper bags, the loss of viability was rather rapid as compared to those kept in other packing material discussed above. The brown paper bags which put some resistance to moisture movement from environment to seeds under high humidity condition were superior to open mouth bottle. The loss of viability in seeds kept in open mouth bottle kept in refrigerator was rapid in the species under study, because of high humidity which prevail in refrigerator (Pedra, 1936; Barton, 1954; 1961; Rengifo and Pfort, 1977; Bhattacharya and Dutta 1980).

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Effect of Colchicine on Morphological and Cytological Parameters of *Trigonella foenum graecum* L.

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Seeds of *Trigonella foenum graecum* L. were treated with different concentration of colchicines for five (5) hours. The effect of colchicines were studied on seed germination, seedling growth, seedlings survival rate and on mitotic chromosomal behavior of the plant. The studies revealed that the germination percentage, seedling growth and survival percentage decreases with the increase dose of colchicines. The cytological studies showed that chromosomal stickiness, fragmentation, chromosome bridge, laggard, ring formation and multipolarity are common effect of colchicines. Percentage of abnormalities increases with increase dose of colchicines and it was found to be maximum at 0.3% colchicine which was recorded as 14%.

Keywords : Medicinal plant, Colchicine, Morphology, Cytology.

INTRODUCTION

Trigoenella foenum graecum L. is an important medicinal plant and used in medicine from ancient period of time. The plant belongs to the family legumenoceae and is a small annual herb. The usefull part of the plant is seeds and used as condiment. The seeds are extensively used in indigestion, diabetes, as a tonic and to prevent hair falling off. The seeds are also rich source of protein, carbohydrate and oil droplet. The somatic number of the species is 2n = 12.

Application of vital and beneficial mutation is an important method of plant breeding in terms of potential adaptability, superior genotype and biochemical composition. Mutagenic agents have been successfully employed by many workers in several lines to induce variation. Raj *et al.,* (1972) reported cytological observations of *Oryza sativa* L. Kumar and Mishra (2006) studied genetic variability of *Vigna mungo* L induced by gamma rays. Novak and Mickie (1987) stated that more than eight hundred varieties of plants have been released as commercial varieties by using mutation breeding. For past few years large number of chemical mutagens have been extensively used and capable of producing mutation. The chemical mutagen colchicines is most effective on physical, chemical and cytological parameters and ultimately affect on yield phenotype of the plant (Majumder, 1991). The degree of cytological abnormalities either mitosis or meiosis is an useful criteria for any crop improvement programme and find out the potency of mutagen and its sensitivity for particular genotype. Researches with colchicines indicate that in certain plants progress in the next 20 years may match that of past 200 years (Chopra. 1985). The present work have been carried out to find out the relative effects of colchicine on cytomorphological characters of *Trigonella foenum graecum* L.

MATERIAL AND METHODS

The healthy seeds of *Trigoenella foenum graecum* L. are selected and treated with freshly prepared aqueous solution of five different concentrations (0.2 - 1%) of colchicines for five (5) hours. The seeds were then thoroughly washed with distilled water and allow to germinate on moist filter paper in a petridish. Observation were made on germination percentage, seedling height, percentage of abnormal seedling, seedling survival rate and mitotic chromosomal behaviour of treated and controlled plants. For cytological studies the root tips were collected from germinating seeds and fixed in Carnoy's fixative (1:3) and squash preparation was made in 2% aceto carmine solution (Sharma and Sharma 1990) and observed under microscope. Cells from each treatment as well as control population were studied.

RESULTS AND DISCUSSION

Seed germination percentage was recorded from 2nd day until no further germination was observed. Germination percentage of *Trigoenella foenum graecum* L. is 98% in control. Germination percentage decreased and showed delayed germination with the increasing dose of colchicines. Among the treated seeds highest germination was recorded at 0.2% colchicines and lowest was at 1% which was 86% and 36% respectively (Table-1). Percentage of germination inhibition was 8.5% and 61.7% with 0.2% and 1% colchicines respectively Kurobane *et al.*, (1979) reported poor seed germination in gamma irradiated Barley seeds which was due to delayed physiological and biological process.

Seedling height were measured on 10th days of seedling emergence & were recorded as 10.2 cm and 4.8 cm in control

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Table 1 : Effect of Colchicine on seed germination & seedling growth of *Trigonella foenum graecum* L.

Treatment (%)	Germination Rate (%)	Seedling height (cm)	Abnormal seedling		Survival rate (%)
			Chlorophyll Mutant (%)	Seedling without radicle (%)	
Control	98	10.2 ± 0.04	-	-	98.1 ± 0.05
0.2	86	9.0 ± 0.04	-	-	84.6 ± 0.21
0.4	60	8.6 ± 0.06	1.5 ± 0.23	1.0 ± 0.10	56.9 ± 0.19
0.6	51	5.4 ± 0.03	2.4 ± 0.18	3.0 ± 0.22	28.1 ± 0.31
0.8	47	4.9 ± 0.02	2.5 ± 0.18	3.4 ± 0.21	-
1.0	36	4.8 ± 0.03	-	4.1 ± 0.19	-

Table 2 : Percentage of chromosomal abnormalities of colchicines treated *T. foenum graecum* L.

Traetmant (%)	Interphase binucleate cell	Stickiness	Laggard	Unequal separation	Multipolarity	Chromosome ring	Total Abnormlities %
Control	-	-	-	-	-	-	-
0.2	-	0.41	0.27	0.34	-	-	1.02
0.4	-	1.23	0.76	0.91	-	-	2.90
0.6	0.84	1.83	1.92	1.34	1.75	1.65	9.33
0.8	0.96	3.46	2.13	1.86	1.94	1.10	11.45
1.0	1.01	3.67	2.16	2.01	2.57	1.77	13.19

and 1.0% colchicine treated plant respectively. The seedling growth was reduced with increase dose of colchicines and no further growth was found beyond 0.6% colchicines (Table - 1). Blixt (1978) stated that the reduction of seedling growth is due to the gross injury at the cellular level due to gene mutation or chromosomal aberration. Hussain & Siddqui or 1997 also stated that chemical mutagen induces various biological damages and it is depent on dose. Seedling survival percentage was highest at 0.2% colchicines and recorded 84.6%. While the value reduced to 28.1% by 0.6% colchicines. Abnormal seedlings without radical were common occurrence in treated plants.

The dividing untreated root tip cells of *Trigonella foenum graecum* L. revealed that the chromosome number is 2n=12. The treated cells showed chromosomal abnormalities and were presented in Table - 2. The frequnecy of abnormalities increase with increase colchicine concentration and it was found to be highest in 1% which was 13.19%. The common chromosomal abnormalities were chromosomal stickiness, fragmentation, chromatinbridge, unequal separation, movement of entire chromosome toward one pole multipolarity etc. in metaphase and anaphase. Multinucleate and persistent nuclear condition was reported by Natarajan (1958) in Maize and by Siddiq (1964) in Sorghum following radiation and colchicine treatment respectively.

From the present studies it is evident that colchicine is effective on cytomorphological parameters of *Trigoenella foenum graecum* L. At higher concentration it causes germination and growth inhibition but at suitable concentration it can be use to create variability for further plant improvement programme.

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Effect of Water Stress on Seed Germination and Seedling Growth in *Vigna radiata* L. Wilczek

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A laboratory experiment was conducted during 2007, 2008 and 2009 to evaluate 19 diverse *Vigna radiata* L. Wilczek (greengram) genotypes for seed germination and seedling growth under different levels of water stress. The results revealed a decline in seed germination percentage and seedling growth in all the genotypes with increasing levels of water stress from -3.0 bars to -10.0 bars. A drastic reduction in seed germination percentage and seedling vigour was noticed at water stress beyond -5.0 bars irrespective of the genotypes. Among the genotypes, SGC-20 and Pratap maintained significantly higher germination percentage and seedling vigour at all the levels of water stress and these two genotypes may be considered as stress (drought) tolerant, particularly during seed germination and early seedling growth stage.

Keywords : Germination, Genotypes, Seedling growth, Water stress.

INTRODUCTION

Vigna radiata L. Wilczek is the third most important pulse crop in India. Its national average productivity is very low. Lack of moisture is one of the important causes of lower productivity of greengram as the crop is generally cultivated under rainfed condition and the crop suffers from intermittent and periodic moisture stress at critical stages of crop growth (Pannu and Singh, 1987).

In the state of Assam, *summer* greengram is sown in the middle of March and the crops suffer from moisture deficit during germination and early vegetative phase. Water stress affects cell water potential, movement of stomata, rate of photosynthesis, metabolism and affecting the yield (Ripley *et al.* 2007). Crop cultivars show differential tolerance to soil moisture stress occurring at different growth stages. On the other hand the water potential that prevents seed germination is critical for a species and studies on this aspect would furnish valuable information for agricultural workers (Levitt, 1972). Such studies have been primarily confined to cereal crops and little research works have been reported on greengram. Therefore, the present investigation has been planned with an objective to study the genotypic variability of germination and seedling vigor under stimulated moisture stress in a set of 19 diverse greengram genotypes.

MATERIAL AND METHODS

The experiment was conducted in the laboratory of the Department of Crop Physiology, B N College of Agriculture, Assam Agricultural University, Biswanath Chariali during 2007, 2008 and 2009 in a factorial experiment arranged in completely randomized design with three replications to evaluate 19 diverse greengram genotypes for germination and seedling growth under different levels of water stress. The genotypes selected for the study were Pratap, K-851, AAU-34, SP-206, SP-201, SP-16, SP-10, KM 5-168, KM 5-160, ML-131, KM 6-133, SP-215, KM 6-134, SP-14, KM 6-130, SGC-16, SGC-2, SGC-6 and SGC-20.

Polyethylene glycol, 6000 (PEG-6000) was used to create different levels of water stress. PEG-6000 was dissolved in 100 ml. of distilled water in concentrations of 11.5, 19.6, 23.5 and 28.9 g. separately to prepare osmoticum solutions of -3.0, -5.0, -7.5 and -10.0 bars water potential respectively, following the method of Hadas (1976). Distilled water was used as control. Hundred (100) number of surface sterilized, uniform sized seeds were placed in petridish with filter papers that were kept moistened uniformly with the above mentioned solutions. The studies were carried from 15th. March of each year till completion as March 15 is the normal sowing time of *summer* greengram in Assam. The petridishes were kept in laboratory condition under normal light and room temperature.

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Seed germination was recorded at a fixed time everyday up to 11 days after sowing. The criterion for laboratory seed germination was taken as the emergence of 2 mm radicle out of the seed coat. At 11 days after sowing, ten seedlings were randomly selected from each petridish and length of radicle and plumule were taken and the averages were computed. The data of three years were pooled and subjected to analysis of variance as per the method of Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

A significant difference was observed with regard to seed germination percentage among the genotypes (Table 1). Germination percentage decreased with increasing levels of moisture stress from -3.0 to -10.0 bars irrespective of the genotypes. At all the levels of stress, higher seed germination percentage was recorded in the genotype SGC-20 followed by Pratap. In control, Pratap recorded higher seed germination percentage followed by AAU-34 and the lower value was recorded in the genotype, KM 6-134. SGC-20 and Pratap recorded seed germination percentage of 34.33% and 31.89%, respectively even at -10.0 bars, whereas most of the other genotypes failed to germinate at this extreme level of stress. The seeds of the genotype ML-131 could not germinate even under -7.5 bars water potential.

Similar result was reported by Goswami (1991) in rice. The decrease in seed germination with increasing levels of stress may be attributed to the increasing solute potential, which was responsible for reduction in seed germination percentage primarily by lowering the rate of imbibition (Field and Gregory, 1989).

A significant reduction in radicle and plumule length was observed with increasing levels of moisture stress from -3.0 up to 10.0 bars irrespective of the genotypes (Table 4). There was a drastic reduction in both radicle and plumule length beyond -5.0 bars. Up to -5.0 bars, the plumule length was more as compared to radicle length which was just reverse at the extreme levels of stresses, *i.e.*, at -7.5 bars and -10.0 bars. At all the levels of stresses, the genotypes SGC-20 recorded significantly higher radicle length followed by Pratap. In control, the genotype KM 6-130 followed by KM 6-133 possessed higher radicle length and the lowest values were recorded in the genotype SP-215. At -3.0 bars, the genotype SGC-20 maintained significantly higher plumule length followed by KM 6-134, while the lowest value was recorded in SP-201. The genotype SGC-20 followed by Pratap possessed higher plumule length under -5.0 and -7.5 bars and it was at par under the extreme level of stress, *i.e.* -10.0 bars. It was observed that at 7.5 bars and -10.0 bars, most of the genotypes could produce the radicle only and were devoid of plumule which is a case of imbalanced germination. Lallu and Dixit (2003) reported that germination percentage, root length, shoot length and speed of germination were reduced significantly with increasing levels of stress in mustard and they also observed differential genotypic tolerance, which is in agreement

Table 1 : Germination (%) under different levels of water stress in greengram.

Genotypes	Control	-3.0 bar	-5.0 bar	-7.5 bar	-10.0 bar	Mean
Pratap	93. 89	82.78	64.11	47.33	31.89	64.00
K- 851	83.08	55.15	50.44	40.33	5.67	46.94
AAU - 34	92.44	82.89	63.33	33.33	7.33	55.67
SP- 206	86.77	80.11	60.22	17.34	0.00	48.87
SP- 201	83.44	78.22	21.32	16.00	2.00	40.20
SP- 16	83.55	63.56	41.45	13.54	0.00	40.54
SP- 10	83.89	75.22	67.33	24.11	9.56	53.15
KM 5-168	82.66	80.44	41.55	31.11	0.00	47.15
KM 5-160	85.44	62.11	53.33	32.78	18.78	50.40
ML-131	89.55	54.67	40.44	0.00	0.00	36.93
KM 6-133	91.77	79.01	53.55	35.67	0.00	52.62
SP- 215	89.56	73.33	50.22	25.00	0.00	49.40
KM 6- 134	81.22	63.70	51.44	31.00	0.00	45.88
SP- 14	85.45	62.89	58.30	24.89	5.33	46.97
KM 6- 130	87.33	68.22	46.66	30.22	6.77	47.58
SGC- 16	81.33	61.11	50.22	40.56	0.00	46.96
SGC- 2	86.45	59.37	48.44	21.33	0.00	43.12
SGC- 6	84.56	71.00	60.67	31.33	24.00	57.46
SGC- 20	91.12	84.00	65.19	52.33	34.33	64.40
Mean	**86.41**	**70.87**	**52.04**	**29.11**	**7.09**	------
	S. Ed.	C D (5%)				
Variety (V)	0.52	1.04				
Treatment (T)	0.28	0.65				
V X T	1.15	2.31				

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Table 2 : Radicle and plumule length (cm) under different levels of water stress in greengram at 11 days after sowing.

Genotypes	Radicle length (cm)						Plumule length (cm)					
	Control	–3.0 bar	–5.0 bar	–7.5 bar	–10.0 bar	Mean	Control	–3.0 bar	–5.0 bar	–7.5 bar	–10.0 bar	Mean
Pratap	5.37	4.53	4.03	1.55	1.00	**3.30**	12.12	5.51	4.00	1.23	1.00	**4.77**
K- 851	4.20	3.89	2.89	1.07	0.00	**2.41**	10.85	3.23	2.83	0.00	0.00	**3.38**
AAU - 34	5.51	3.67	1.45	0.97	0.00	**2.32**	12.65	5.40	4.34	0.00	0.00	**4.48**
SP- 206	4.36	3.50	1.50	0.00	0.00	**1.87**	8.32	2.01	1.78	0.00	0.00	**2.42**
SP- 201	4.10	2.83	2.01	0.78	0.00	**1.94**	7.21	1.89	1.44	0.00	0.00	**2.11**
SP- 16	5.08	2.96	1.16	0.50	0.00	**1.94**	7.56	3.23	2.57	0.00	0.00	**2.67**
SP- 10	4.13	3.00	2.09	0.66	0.00	**1.98**	12.00	3.67	3.23	0.00	0.00	**3.78**
KM 5-168	3.87	2.55	1.21	0.48	0.00	**1.62**	6.78	4.09	3.89	0.00	0.00	**2.95**
KM 5-160	3.89	3.77	3.00	0.33	0.33	**2.26**	7.45	4.78	3.00	1.00	0.33	**3.31**
ML-131	4.01	3.12	1.08	1.01	0.00	**1.84**	7.34	3.32	3.87	0.00	0.00	**2.91**
KM 6-133	6.25	2.96	2.02	0.00	0.00	**2.25**	12.11	3.88	2.43	0.00	0.00	**3.68**
SP- 215	3.57	3.33	1.11	0.00	0.00	**1.60**	8.89	2.50	1.67	0.65	0.00	**2.74**
KM 6- 134	4.03	3.08	2.12	0.00	0.00	**1.85**	7.02	6.00	5.00	1.22	0.43	**3.93**
SP- 14	5.23	4.06	1.04	0.50	0.00	**2.17**	8.12	5.45	2.45	0.50	0.00	**3.30**
KM 6- 130	6.31	4.43	3.34	1.00	0.56	**3.13**	5.86	4.34	2.07	0.00	0.00	**2.45**
SGC- 16	5.08	3.09	2.42	1.21	0.33	**2.43**	5.23	4.12	2.09	0.55	0.00	**2.40**
SGC- 2	5.64	3.57	2.23	0.78	0.00	**2.44**	8.78	5.78	5.01	0.00	0.00	**3.91**
SGC- 6	4.04	3.06	2.04	1.13	0.00	**2.05**	6.89	4.33	3.10	0.50	0.00	**2.96**
SGC- 20	5.56	4.67	4.11	1.58	1.02	**3.39**	12.23	6.51	5.32	1.25	1.00	**5.26**
Mean	**4.75**	**3.48**	**2.15**	**0.71**	**0.18**	**--------**	**8.81**	**4.21**	**3.16**	**0.36**	**0.14**	**------**
		S. Ed	C D(5%)					S.Ed	CD(5%)			
Variety (V)		0.49	1.00				V	0.51	1.02			
Treatment (T)		0.26	0.52				T	0.26	0.52			
V X T		1.18	2.37				VX T	1.15	2.30			

with the results of present findings. Similar results were reported by Goswami and Baruah (1994); Anaytullah *et al.*, (2007) Das and Kalita (2010) in rice.

Although a significant differential tolerance was exhibited by the genotypes with respect to germination and early seedling growth, the genotypes SGC-20 and Pratap possessed some degree of tolerance particularly during germination and seedling growth stage. These genotypes may be recommended for early sowing in the state of Assam under moisture deficit condition in *summer* season.

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Antioxidant Activity of Various Leaf Extracts of *Cajanus cajan* L.

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The antioxidant activity of petether, chloroform, methanol, ethanol and aqueous extracts of the leaves of *Cajanus cajan* L. were determined by ferric reducing power, ferric thiocynate (FTC) method and DPPH radical scavenging assay. Ethanol showed the highest extract yield (12.29%) and total phenolic content (179.46 ± 0.71 mg/g gallic acid) among all the solvents. The ethanolic extract showed highest reducing power at 100µg/ml than standard BHA. The ethanolic extract exhibited highest antioxidant activity than methanol, aqueous, petether and chloroform extract. The DPPH assay showed that the ethanol and methanol extracts have comparatively admirable inhibition capacity when compared to the standard BHA. The present study showed ethanol to be a potential extractant for the natural antioxidant present in *Cajanus cajan*.

Keywords : Antioxidant activity, *Cajanus cajan*, DPPH, FTC.

INTRODUCTION

Antioxidant research is an important topic in the medical field as well as in the food industry. Antioxidants protect other molecules from oxidation, when they are exposed to free radicals and reactive oxygen species, which have been implicated in the etiology of many diseases and in food deterioration and spoilage (Halliwell and Gutteridge, 1992, Kasaikia *et al.*, 1997, Farombi, 2000, and Koleva *et al.*, 2000). Natural antioxidants such as α- tocopherol and L-ascorbic acid are used because they are seen as being safer and causing less adverse reactions but their antioxidant activities are however lower than those of synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxyl toluene (BHT). Hence there is need for safe, economic antioxidants with high activity from natural sources to replace synthetic chemicals. A profile of the chemical composition of the plant together with knowledge of its antioxidant activity will give a fair estimate of its therapeutic potential (Akinmoladun *et al.*, 2007).

Cajanus cajan L is a perennial member of the family Leguminosae, commonly known as pigeonpea or red gram in English and arhar in Hindi. It is one of the most important dietary legume crop predominantly grown in the tropical regions. India contributes about 90% of the world production of *Cajanus cajan* L. compared with other grain legumes, pigeonpea ranks only sixth in area and production, but it is used in more diverse ways than others (Chakraborty *et al.*, 2007). The extracts or components of pigeonpea are commonly used all over the world for the treatment of diabetes, dysentery and hepatitis (Grover *et al.*, 2002). Now a days, these leaves are used for the treatment of wounds, aphtha, bedsores and malaria as well as diet-induced hypercholesterolemia etc (Aiyeloja and Bello *et al.*, 2006 and Luo *et al.*, 2008). Chemical constituents investigations have indicated that pigeonpea leaves are rich in flavonoids, stilbenes which are considered responsible for the beneficiaries of the leaves on human health (Zu *et al.*, 2006 and Zheng *et al.*, 2007). The present study focus on the identification of known or novel bioactive compounds from the plant that may provide the basis for value added products for the pigeonpea industry. The main objective of the work is to evaluate the antioxidant potential of various leaf extracts of *Cajanus cajan* L.

MATERIAL AND METHODS

Plant collection and extraction : The leaves of *Cajuns cajan* L. varieties Maruti (ICP-8863) were collected from the field of Gulbarga District in Karnataka, India. The leaves were dried under shade and made into fine powder using pestle and mortar. Five hundred grams of the powdered plant material was subjected to the soxhlet successive extraction method (60-80°C) using about 2.5 liters of petroleum ether, chloroform, methanol, ethanol and distilled water (aq) in the order of increasing polarity of solvent for a period of 18h. The extracts obtained were dried in vacuum at 40°C and then weighed.

Total phenolic determination : The total phenolic content was determined mixing 0.5ml of the sample extracts with 2.5ml 10% Folin-Ciocalteau reagent (v/v) and 2.0 ml of 7.5% sodium carbonate was subsequently added. The reaction mixture was incubated at 45°C for 40 min and the absorbance was measured at 765nm using a spectrophotometer. Gallic acid was used as standard phenol (Malick and Singh, 1980).

Antioxidant activity :

Determination of reducing power: The reducing power of

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the prepared extracts was determined using Ferricyanide trichloroacetic acid method according to Oyaizu (1986). Mean values from the independent samples were calculated for each extract.

Ferric thiocynate (FTC) method: The antioxidant activities of extracts were determined by Ferric thiocynate method (Mistuda *et al.*, 1996). The sample mixture contain 0.5ml of extract, 2.5ml of linoleic acid emulsion and 2ml phosphate buffer (0.05M pH 7.0) in a test tube and incubated in darkness at 37°C. The amount of peroxide was determined by reading absorbance at 500nm after coloring with $FeCl_2$ and Thiocynate at intervals during incubation. BHA was used as standard antioxidant.

DPPH radical scavenging assay: The potential antioxidant activity of extract were determined on the basis of scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by the method of Blois (1958). 0.1ml of sample at various concentrations was mixed with 2.9 ml of methanolic DPPH (60µM) solution. The mixture was left in the dark for 30min and absorbance was measured at 517nm.

All the experimental triplicate results were analysed by one way ANOVA using SPSS package (version 10).

RESULTS AND DISCUSSION

The extraction yield and phenolic content of the extracts of *Cajanus cajan* L. leaves are shown in Table-1. The ethanolic extract showed the significantly ($P<0.05$) highest extraction yields (12.29%) and total phenolic content (179.46 ± 0.71 mg/g gallic acid) among all solvents. The extraction yield of different solvents varied from 6.2% to 12.29 and could be ranked from high to low *i.e.,* ethanol > methanol > petether > chloroform > aqueous extracts. Herodez *et al.*, (2003) reported that the percentage of extraction yield will increase with the ratio of solvents, temperature and sample extraction. The antioxidant activity of phenolics compounds is reported to be mainly due to their redox properties. This can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Galato *et al.*, 2001, Zheng and Wang, 2001).

The ferric reducing power of petether, chloroform, methanol, ethanol and aqueous extract are presented in Table2. The reducing power is to measure the reductive ability of antioxidant and is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of the sample extract. The data showed that all the extracts increased their reducing ability when its concentration was increased. This result is similar to that reported by Gulcin *et al.*, (2003) and Noriham *et al.*, (2004) in *Pimpinella anisum* seeds extracts and four types of Malaysian plants. The reducing power was significantly ($P<0.05$) superior in ethanolic extract fallowed by methanol, aqueous, chloroform and petether. At 100µg/ml the reducing power of ethanolic extract have higher ability than standard BHA to reduce Fe^{3+} to Fe^{2+}. The ability to reduce Fe^{3+} may be attributed from hydrogen donation from phenolic compounds (Shimada *et al.*, 1992) related to the presence of reduced agent (Duh, 1998).

The antioxidant activity of the petether, chloroform, methanol, ethanol and aqueous extract of *Cajanus cajan* L. leaves determined by ferric thiocynate (FTC) are presented in Table-3. FTC method was used to determine the amount of peroxide formed and that react with ferrous chloride ($FeCl_2$) pigment. In this method the concentration of peroxide decreases as the antioxidant activity increases. The antioxidant activity of ethanolic extract is significantly ($P<0.05$) higher than the other extracts. The antioxidant activity of ethanol extract (79.62%) was very close to the standard BHA (80.06%) at 100µg/ml. It was observed that the extract exhibited strong activity with increase in polarity, indicating that polyphenols or flavonoids may play an important role in the antioxidant activities. The present findings are in agreement with the report of Tepe *et al.*, (2005).

The DPPH scavenging activity of petether, chloroform, methanol, ethanol and aqueous extracts of *Cajanus cajan* L. leaves are shown in the Table-3, the results are statistically significant at $P<0.05$. Extracts of ethanol and methanol showed admirable scavenging activities compared to BHA at 100µg/ml. However petether, chloroform, aqueous extracts showed lower scavenging activity than BHA. Arambewela *et al.*, (2006) reported that cold ethanolic extract of the leaves of *Piper betle* showed promising antioxidant activity compared to that of synthetic antioxidant butylated hydroxyltoluene.

From the present investigation it is evident that, the solvents extracts significantly affected the yield, total phenolics, reducing power and antioxidant activity. It also revealed that ethanolic extract showed highest yield, phenolic content and antioxidant activity. Thus the results indicate that selective extraction from natural materials, by an appropriate solvent, is important for obtaining fractions with high antioxidant activity. Ethanol could be the optimal solvent for extraction of antioxidant compound in *Cajanus cajan* L. leaves.

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Table 1 : Extraction yields and total phenolic content of various leaf extracts of *Cajanus cajan* L.

Extract	Yield of Extracts (%)	Total Phenolics (mg/g gallic acid equivalent)
Petether	10.4%	5.25 ± 0.003
Chloroform	6.2%	7.61 ± 0.009
Methanol	8.6%	8.25 ± 0.001
Ethanol	12.29%	9.01 ± 0.006
Aqueous	5.0%	3.74 ± 0.007

Each value is expressed as mean ± S.D (n=3) and statistically significant at $P<0.05$

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Table 2 : Reducing power of various leaf extracts of *Cajanus cajan* L.

Extract	Reducing power OD 700					CD Values
	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
Petether	0.322 ± 0.006	0.370 ± 0.012	0.463 ± 0.005	0.480 ± 0.001	0.51 ± 0.002	0.3707
Chloroform	0.130 ± 0.015	0.220 ± 0.013	0.418 ± 0.004	0.468 ± 0.003	0.533 ± 0.017	0.1065
Methanol	0.415 ± 0.000	0.678 ± 0.008	0. 875 ± 0.001	1.101 ± 0.007	1.175 ± 0.012	0.7344
Ethanol	0.668 ± 0.002	1.182 ± 0.001	1.281 ± 0.0012	1.603 ± 0.005	1.607 ± 0.009	0.8170
Aqueous	0.121 ± 0.002	0.827 ± 0.002	0.830 ± 0.0013	0.817 ± 0.006	1.086 ± 0.006	0.7917
BHA	0.510 ± 0.001	0.732 ± 0.001	1.110 ± 0.001	1.418 ± 0.002	1.575 ± 0.002	0.8815

Each value is expressed as mean ± S.D (n=3) and statistically significant at P<0.05 CD- Critical Difference.

Table 3 : Antioxidant activity of various leaf extracts of *Cajanus cajan* L. by FTC method.

Extract	% of Inhibition					CD Values
	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
Petether	9.32 ± 0.002	10.34 ± 0.006	24.30 ± 0.004	25.11 ± 0.050	33.88 ± 0.005	4.2682
Chloroform	12.4 ± 0.002	12.80 ± 0.001	19.3 ± 0.002	22.56 ± 0.001	30.34 ± 0.003	3.5907
Methanol	35.36 ± 0.000	39.36 ± 0.001	45.15 ± 0.002	46.23 ± 0.002	54.86 ± 0.001	3.5853
Ethanol	46.85 ± 0.015	61.34 ± 0.012	68.92 ± 0.015	69.8 ± 0.020	79.62 ± 0.012	4.5937
Aqueous	23.81 ± 0.006	27.81 ± 0.012	34.21 ± 0.015	41.25 ± 0.003	45.59 ± 0.007	3.960
BHA	66.65 ± 0.002	68.28 ± 0.003	72.44 ± 0.002	73.5 ± 0.001	80.06 ± 0.001	3.010

Each value is expressed as mean ± S.D (n=3) and statistically significant at P<0.05 CD- Critical Difference.

Table 4 : DPPH Scavenging assay of various leaf extracts of *Cajanus cajan* L.

Extract	% of Inhibition					CD Values
	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
Petether	12.80 ± 0.005	28.19 ± 0.009	26.87 ± 0.007	32.12 ± 0.008	42.08 ± 0.007	4.2795
Chloroform	10.81 ± 0.001	15.44 ± 0.002	22.43 ± 0.003	47.66 ± 0.003	55.03 ± 0.001	5.863
Methanol	23.2 ± 0.001	34.2 ± 0.001	68.0 ± 0.002	80.8 ± 0.002	95.2 ± 0.002	7.283
Ethanol	32.46 ± 0.009	43.38 ± 0.007	58.4 ± 0.012	66.8 ± 0.009	95.3 ± 0.009	6.463
Aqueous	8.02 ± 0.003	9.27 ± 0.006	15.08 ± 0.017	18.4 ± 0.006	25.4 ± 0.006	3.5034
BHA	52.73 ± 0.004	68.91 ± 0.001	87.01 ± 0.002	90.2 ± 0.002	95.8 ± 0.001	5.548

Each value is expressed as mean ± S.D (n=3) and statistically significant at P<0.05 CD- Critical Difference.

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Effect of Salinity on Growth of *In Vitro* Plantlets in Sugarcane

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To develop salt tolerant sugarcane lines, effect of salinity on growth, number of internodes and internodal length was carried out in three sugarcane varieties *viz* Co. 86032, Co. 7714 and Co. 671. The plantlets regenerated from the salt treated calli were transferred into the soil simulated with salinity levels *viz*, $NaHCO_3$, Na_2SO_4, $CaCl_2$ and $MgCl_2$ in proportion of 2:1:1 of chlorides, carbonates and sulphates respectively. Salinity affected growth adversely in all varieties. However least adverse effect was recrorded in variety Co 86032 followed by Co 671 and Co 7714. Among the three varieties Co 86032 exhibited highest salinity tolerance.

Keywords : Salt tolerance , Salinity, Growth, Sugarcane.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is one of the most important sugar crop of India. Salt tolerance is a widespread agricultural problem (Gosal and Bajaj, 1984) and the presence of excessive concentration of salts in soil severely inhibits plant growth (Hunsigi, 1993). The development of salt tolerant crop cultivars has been considered to be an efficient and economic means of overcoming salinity problems. The recent development in the *in vitro* technology offers a meaningful tool for determining the tolerance and also for screening and developing the salt tolerant genotypes. Pesent investigation was carried out *in vitro* to study the effect of salinity on growth, number of internodes and internodal length of sugarcane plantlets.

MATERIAL AND METHODS

The effect of salinity on plant height, number of internodes and internodal length was studied in three sugarcane varieties *viz*., Co. 86032, Co. 7714, and Co. 671. The experimental work was carried out by preparing microplots in the field. The microplots developed in three replicates were bordered with thick polythene placed 45 cm into the ground and extended 15 cm above to avoid seepage of salts (Shalhevt and Bernstein, 1968). Two salinity levels such as 4 and 6 EC was simulated in the microplots by adding the salts *viz*., $NaHCO_3$, Na_2SO_4, $CaCl_2$ and $MgCl_2$ in proportion of 2 : 1 : 1 of chlorides, carbonates and sulphates respectively in the soil (More and Malewar, 1988). The salts was added in the soil till the particular salinity were obtained. The previously *in vitro* salt treated plantlets were hardened for forty days in green house and transferred in to the microplots in the Factorial Randomized Block Design. Observations on the effect of salinity on growth, number of internodes and internodal length was recorded after four months of planting.

RESULTS AND DISCUSSION

The data on growth, number of internodes and internodal length of the selected clones in the simulated salinity levels presented in Table 1, 2 and 3. The results reveal that for all the characters among the variety and treatment differences were significant. Salinity affected growth adversely in all varieties. However least adverse effect was recorded in variety Co 86032 followed by Co 671 and Co 7714. These results find support from work of Montese-de-Oca *et al.*, (1996), Sharma *et al.*, (1997), Tiwari *et al.* (1997) and Lingle *et al.*, (2000). Thus among the three varieties the clones of the variety Co 86032 exhibited highest salinity tolerance.

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Table 1 : Effect of salinity levels on plant height (cm).

Varieties	Simulated salinity in soil			Mean
	Control	4EC	6EC	
Co. 86032	102.50	80.00	73.33	85.27
Co. 7714	78.33	66.66	50.00	65.00
Co. 671	95.00	73.33	64.16	75.00
Mean	91.94	73.33	62.50	75.92
	Variety	Treatment	Interaction	
S.E. ±.	3.25	3.25	5.64	
C.D. at 5%	9.75	9.75	NS	

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Table 2 : Effect of salinity on number of internodes.

Varieties	Salinity Simulated in soil			Mean
	Control	4EC	6EC	
Co. 86032	9.20	8.73	8.53	8.82
Co. 7714	8.00	7.66	6.53	7.40
Co. 671	9.00	8.46	7.46	8.31
Mean	8.73	8.26	7.51	8.17
	Variety	Treatment	Interaction	
S.E. ±	0.375	0.375	0.650	
C.D. at 5%	1.123	1.123	NS	

Table 3 : Effect of salinity on internodal length (cm).

Varieties	Simulated salinity in soil			Mean
	Control	4EC	6EC	
Co. 86032	8.80	8.40	8.12	8.44
Co. 7714	6.14	5.66	5.53	5.77
Co. 671	8.02	7.49	6.73	7.41
Mean	7.65	7.18	6.79	7.21
	Variety	Treatment	Interaction	
S.E. ±	0.24	0.24	0.41	
C.D. at 5%	0.718	0.718	NS	

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Impact of Integration of Fly Ash with Vermicompost on Black Cotton Soil Enzyme Activities and Mulberry (*Morus alba* L.) Leaf Yield and Quality

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A field experiment was conducted at Research Extension Centre, Vikarabad to study the impact of integration of fly ash (FA) with vermicompost (VC) and their combinations on black cotton soil enzyme activities and mulberry leaf yield and bio-assay of leaf quality through silkworm rearing. The experimental soil was black cotton soil in texture, slightly alkaline in reaction (pH 7.9), non-saline (EC 0.29 dS/m), low in available N(209 Kg/ha), available phosphorous (8.5 Kg P_2O_5/ha/Kg), medium in available potassium (179 Kg/ha K_2O), medium in available sulphur (8.3 mg/Kg). The highest mulberry leaf yield, leaf moisture content and leaf moisture retension capacity were recorded due to the combined effect of the fly ash (10 MT/ha/yr) and vermicompost (10 MT/ha/yr). In case of silkworm rearing, the minimum larval duration, the maximum 10 larval weight, effective rate of rearing, single cocoon weight, single shell and shell ratio were recorded due to the combined effect of fly ash (10 MT/ha/yr) and vermicompost (10MT/ha/yr). The combined application of fly ash (15 MT/ha/yr) and vermicompost (10 MT/ha/yr) has resulted in highest urease, dehydrogenase and cellulase activities at 60 DAT (4.81µg of NH_4^+ released/g soil/h, 5.47 mg of TPF produced/g soil/d and 2.98 mg of glucose released / g soil/d than other stages. The acid and alkaline phosphatase activities were not influenced by fly ash levels at all stages *viz*, 30, 60 DAT and at (70th day) leaf harvest. However, it was significantly influenced by vermicompost application.

Keywords : Fly ash, Vermicompost, Mulberry leaf yield, Leaf bio-assay, Soil enzymes.

INTRODUCTION

Fly ash is a major solid industrial waste in India. It is a by-product of thermal power stations, where electricity is produced by firing finely powdered coal. Huge quantity of fly ash is produced every day at every thermal power station in the country. This huge quantity of fly ash is dumped in ash disposed areas which creates the land degradation problems and may cause potential environmental hazards in spreading diseases and leaching of unwanted chemicals in to ecosystem. In an attempt to effectively solve the disposal problem of this enormous solid industrial waste, some efforts have been made to utilize it as an amendment to improve soil and its fertility and for crop production (Sahoo and Kar, 1998). The increase in activities of different enzymes can be attributed to increase in soil microbial activity and favourable soil environment due to application of fly ash and organic manure (Lal *et al.*, 1996).

The enzyme urease involves in the reactions related to breakdown of urea to CO_2, water (H_2O) and NH_4^+. The enzyme dehydrogenase transfers electrons from one to another substance and is involved in degradation of carbohydrates, liquids, etc. By involving water, the enzyme phosphatase breaks 'humus-O-P-OH bond' to produce humus-OH and H_3PO_4, which helps to decompose humus, making P available to plants. The enzyme cellulase breaks celluloses, which are long chain of sugar units. It is important in organic matter decay. The Urease, Phosphatase and cellulase are the extracellular enzymes secreted by soil microorganisms, whereas dehydrogenase enzyme exposed in intact cell. Thus, the amount and activities of these enzymes indicate essentially the biological activity of the soil. Hence, an experiment was conducted to study the impact of integrated use of fly ash with vermicompost on soil enzyme activities (urease, dehydrogenase, acid and alkaline phosphatase and cellulase) of black cotton soil and mulberry leaf yield and quality.

MATERIAL AND METHODS

A field experiment was conducted in black cotton soil during 2005-07 at mulberry farm of Research Extension centre, Vikarabad. The soil of the experimental field was deep,

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moderately drained, clay loam, low in available N (209 Kg/ha), medium in available phosphorus (8.5 P_2O_5 Kg/ha), available potassium (179 Kg/ha) and medium in available potassium (179 Kg K_2O/ha). The soil pH was 7.9 and EC was 0.41 dSm. Well established three years old Victory-1 mulberry variety (V-1 variety) grown in a spacing of (4' + 3') x 2' was used as test crop. The experiment was laid out in split plot design with three replications. The experiment comprised eight treatment combinations with four levels of fly ash (0, 5, 10 and 15 MT/ha/yr) and two levels of vermicompost (0 and 10 MT/ha/yr).The fly ash and vermicompost were applied as per the treatments. All the plots were applied the recommended doses of NPK fertilizers (350 Kg N: 140 Kg P: 140 Kg K /ha/yr) in five split doses per year.

The fly ash was collected from National Thermal Power Corporation (NTPC), Ramagundam, Andhra Pradesh. It contained the nutrients like N (27.5 mg/Kg), P_2O_5 (29.6 mg/Kg), K_2O (17.50 mg/Kg), Ca (7.25mg/Kg), Mg (2.20 mg/Kg), Fe (17.50 mg/g), Mn (3.34 mg/Kg), Cu (0.98 mg/Kg) and Zn (1.83 mg/Kg). The texture of fly ash was silty loam with pH 8.1 and EC 0.37 dS/m. The vermicompost was prepared from sericulture waste in the mulberry farm. It contains nitrogen (1.5%), phosphorous (0.8%), potassium (1.0%), calcium (4.40%), copper (27ppm), zinc (278ppm), Iron (7563ppm), magnesium (0.46%), and manganese (475ppm). The fly ash and vermicompost were applied as substitute for farm yard manure (FYM), which was recommended (20 Mt/ha/yr) in two split doses for 1st and 3rd crops out of five crops per year. The experimental soil was black cotton soil in texture, slightly alkaline in reaction (pH 7.9) non-saline (EC 0.29 dS/m), low in available N(209 Kg/ha),available phosphorous (8.5 Kg P_2O_5/ha/Kg), medium in available potassium (179 Kg/ha K_2O), medium in available sulphur (8.3 mg/Kg) and sufficient in micronutrient status (Fe 8.60 mg/Kg, Mn 5.53 mg/Kg, Cu 1.09 mg/Kg and Zn 1.06 mg/Kg). The total leaf was assessed by harvesting the leaves from all the plants available in the net treated plot area of 10m x 10m and converted to yield per hectare (Venkataramana *et al.*, 2002). A total ten crops were harvested for two years. All the mulberry leaves on healthy shoots of those plants were harvested at 9.00 A.M and 4 P.M. and used for the recording the leaf moisture and leaf moisture retention capacity, 6 hours after harvest (Jolly and Dandin,1996).The pooled data for ten crops was statistically analyzed (Snedecor and Cochran,1967). Bioassay of leaf was conducted following the improved technology of silkworm rearing (Krishnaswami, 1986) by feeding to the popular multivoltine silkworm hybrid, PM X CSR2, as test hybrid to assess the leaf quality. 300 larvae were maintained in each treatment with three replications. Leaves from respective treatment plots were used for feeding the silkworms till spinning. Larval duration, larval weight, effective rate of rearing(ERR),single cocoon weight, single shell weight and shell ratio were recorded and statistically analyzed (Snedecor and Cochran,1967) at 70 days after treatment(DAT).

At periodical intervals (30, 60 and 70 DAT) the soil enzymatic activities *viz.*, urease, dehydrogenase, acid and alkaline phosphatase and cellulase were analyzed. Urease activity was assayed by quantifying the rate of release of NH_4^+ from the hydrolysis of urea as described by Tabatabai and Bremner (1972) but with some modifications as suggested by Sankara Rao (1989). Dehydrogenase activity was assayed by quantifying the mg of TPF (2, 3, 5-tri-phenyl formazon) produced and expressed as g soil/d as described by Casida *et al.* (1964). The acid and alkaline phosphatase activity was assayed by quantifying the amount of p-nitrophenol released and expressed as µg of nitrophenol released/g soil/d as described by Tabatabai and Bremner (1969). Cellulase activity was measured by monitoring the release of reducing sugars using carboxy methylcellulose as substrate as described by Pancholy and Rice (1973).

RESULTS AND DISCUSSION

Mulberry leaf yield : Fly ash and vermicompost and their interactions showed significant effect on mulberry leaf yield (Table 1). The maximum leaf yield (64.50 MT/ha/yr) was recorded due to the combined effect of fly ash (10 MT/ha/yr) and vermicompost (10 MT/ha/yr), which was at par with fly ash (15 MT/ha/yr) and vermicompost (10 MT/ha/yr) (64.00 MT/ha/yr) and has recorded significantly higher leaf yield over other treatment combinations and controls (FA_0 VC_0). The nutrients released to the soil system, conductive to physical environment leading to better loosening, decreasing compactness and increasing the aeration of clay soil, root activity and nutrient absorption and consequent complementary effect would have resulted in higher mulberry leaf yield. Similar results were also obtained by Aravinda Kumar *et al.*, (1998) and Jayabal *et al.* (2000) on growth and yield of rice due to associative effect of fly ash and organic manure, FYM, as well as individual effect of fly ash. Ranganathan and Perumal (1995) reported that cabbage dry matter yield was increased due to application of vermicompost. Das *et al.* (2002) reported that due to influence of vermicompost the mulberry leaf yield and its attributes significantly increased as compared to FYM and recommended dose of chemical fertilizers. Sreenivasulu Reddy *et al.* (2001) also indicated that amendment of soil with vermicompost and FYM registered significantly higher leaf yield in mulberry. Nambiar and Gosh (1989) reported that the beneficial effect of organic manures in terms of sustained production could be attributed to the enhanced biological activities in the rhizosphere, improved soil structures and enhanced availability of nutrients.

Leaf moisture percentage : The highest leaf moisture percentage (73.50%) was registered due to combined effect of fly ash @10 MT/ha/yr and vermicompost @10 MT/ha/yr (FA_{10} VC_{10}) treatment, which was at par with the associative effective of fly ash @15 MT/ha/yr and vermicompost @10 MT/ha/yr ($FA_{15}VC_{10}$) (72.60%) and has recorded significantly higher leaf moisture percentage over other treatment combinations and controls (FA_0 VC_0) could be attributed to the stimulatory effect of the above nutrients in absorption of more soil moisture. Leaf moisture is an indispensable element for mulberry plants and body of silkworms and at the same time, it takes charge for transportation of nutrients in the body of plants and animals. Hence, the moisture content in the leaves may serve as one of the best criteria in estimating their quality (Venkataramana *et al.*, 2002).

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Leaf moisture retension capacity : The maximum leaf moisture retension capacity (79.50%) was recorded by associative effect of fly ash @10 MT/ha/yr and vermicompost @10 MT/ha/yr(FA_{10} VC_{10}), which was at par with the treatment of fly ash @15 MT/ha/yr with vermicompost @10 MT/ha/yr ($FA_{15}VC_{10}$) (78.00%) as compared to other treatment combinations and controls (FA_0 VC_0), is might be due to enhancement of thickness of leaves and ratio of palisade to parenchyma (Hesketh *et al.*, 1985), which facilitate to moisture conservation and net assimilation rate by combined effect of fly ash with vermicompost. Kurtz (1950) found that the formation of wax, which has been considered to play an important role in water conservation of plants, was proportional to the thickness of cuticle. The thickness of the leaf is also physiologically important, since thicker leaves tend to have greater mesophyll surface unit area and hence facilitate to high photosynthetic rate (Nobel and Hartsock 1981).

Bio-assay of leaf quality : Fly ash, vermicompost and their combined effects influenced significantly on larval and cocoon characters (Table 2 and 3). The maximum reduction in larval duration (25.42 days), maximum ten larval weight (50.80 g), effective rate of rearing (97.62%), single cocoon weight (1.965 g), single shell weight (0.406 g) and shell ratio (20.65%), were recorded by fly ash @10 MT/ha/yr with vermicompost @10 MT/ha/yr ($FA_{10}VC_{10}$) treatment, which was at par with the treatment of fly ash @15 MT/ha/yr with vermicompost @10 MT/ha/yr ($FA_{15}VC_{10}$) and has recorded significant reduction in larval duration and improvement in cocoon characters over the other treatment combinations and controls (FA_0VC_0). This is an indication of better leaf quality due to the influence of supplementation of minerals from soil as a result of combined effect of fly ash and vermicompost. Narayanaprakash *et al.*, (1985) opined that the leaf quality is a conductive factor for reduction of larval span (duration), improvement in cocoon characters.

Enzyme Activities : The urease, dehydrogenase and alkaline phosphatase enzyme activities were increased up to 60 DAT which was later decreased at (70 days) harvest. Cellulose, acid and alkaline phosphatase activities were increased up to 30 DAT, which was later decreased by the time 60 DAT and at (70th day) harvest (Tables 4-8).

Urease Activity : Urease activity ranged from 1.78 to 4.50, 1.86 to 4.81 and 0.89 to 2.54 µg of NH_4^+ released/g soil/h at 30, 60 DAT and at harvest, respectively (Table 4). The highest urease activity was recorded with the associative effect of fly ash @ 15 MT/ha/yr and vermicompost @ 10 MT/ha/yr (FA_{15} VC_{10}). The treatment $FA_{15}VC_{10}$ has recorded about 2.52, 2.58 and 2.85 times increase in urease activity at 30, 60 DAT and at harvest (70th day), respectively, over control (FA_0 VC_0) could be the reason that the added readily decomposable fly ash and vermicompost could have increased urease, because they stimulate the growth of urease-producing soil. Askin and Kizilkaya (2005) reported that the urease activity in soils is highly correlated to organic matter(OM), total nitrogen and cation exchange capacity(CEC). Urease catalysis the hydrolysis of urea fertilizer applied to the soil into NH_3 (ammonia) and CO_2. Soil urease originates from plants and micro-organisms found as both intra- and extra-cellular enzymes (Mobley and Hausinger, 1989). The activity of this enzyme in the soils is affected by several factors, such as cropping history, organic matter content of the soil, soil depth, soil amendments, heavy metals and environmental factors (Yang *et al.*, 2006). The stability of this enzyme in soils is affected by several factors as studies shown that extracellular urease associated with the soil organo-mineral complexes is more stable than urease in the soil solution(Burns,1986) and those humus-urease complexes extracted form soil are highly resistant to denaturing agents like extreme temperatures and proteolytic attack(Nannipieri *et al.*, 1976). On the other hand, urease extracted from splants or micro-organisms is rapidly degraded in soil by proteolytic enzyme (Zantua and Bremner, 1977). This suggests that a significant fraction of proteolytic activity in soil is carried out by immobilisation on organic and mineral soil colloids. The increase in urease activity due to application of organic manure alone was reported by Reddy and Chhonkar (1991).

Dehydrogenase Activity : The treatment combination of fly ash @ 15 Mt/ha/yr and vermicompost @ 10 MT/ha/yr (FA_{15} VC_{10}). has recorded about 6.8, 8.9 and 14.4% increase in dehydrogenase activity at 30, 60 and at (70th day) harvest, respectively, over controls (FA_0 VC_0). It ranged from 5.03 to 5.37, 5.02 to 5.47 and 2.70 to 3.09 mg of PTF produced/g soil/d at 30, 60 DAT and at (70th day) harvest, respectively (Table 5). Increased dehydrogenase activity could be due to enhanced organic matter in the soil as a result of associative effect of fly ash and vermicompost (FA_{15} VC_{10}). Klein *et al.*, (1971) reported that the dehydrogenase activity could be used as an index of the metabolic activity of the microbial population in soils. This enzyme is considered to exist as an integral part of intact cells but does not accumulate extracellularly in the soil. Dehydrogenase enzyme is known to oxidize soil organic matter by transforming protons and electrons from substrates to acceptors. These processes are part of respiration pathways of soil micro-organisms and are closely related to the type of soil and soil air-water conditions (Gilnski and Stepniewski,1985). Sriramachnadrasekharam *et al.*, (1997) reported that the increased dehydrogenase activity has resulted due to organic manure / farm yard manure in flooded rice soil. Several authors (Parham *et al.*, 2002; Madejon *et al.*, 2003) observed increased dehydrogenase activity after incorporation of organic residues, indicated an activation of microorganisms following carbon input. Shannon *et al.*, (2002) reported that changes in soil microbiology might occur as a consequence of switching to organic land management.

Acid and Alkaline Phosphatase Activities : The activity of acid and alkaline phosphatase activities were not significantly influenced by fly ash at all stages *viz.*, 30, 60 DAT and at harvest (70th day). However, both the activities were significantly influenced by vermicompost applications and their interaction. Application of vermicompost @ 10 MT/ha/yr has recorded 39.7, 41.0 and 37.6 % increase in acid phosphatase activity at 30, 60 DAT and at harvest (70th day), respectively, over VC_0 (Table 6). Similarly, alkaline phosphatase activity was increased by 3.8, 47.3 and 46.3% due to VC_{10} application at 30, 60 DAT and at harvest (70th day) when compared to VC_0 (Table 7). The

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Table 1 : Effect of fly ash and vermicompost on mulberry leaf yield and leaf moisture content (%) and leaf moisture retension capacity (%).

Fly ash (Mt/ha)	Vermicompost levels (MT/ha/yr)								
	Leaf yield (Mt/ha/yr)			Leaf moisture content (%)			Leaf moisture retension capacity (%)		
	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean
FA_0	24.60	38.50	**31.55**	37.50	35.00	**36.25**	55.0	60.50	**57.75**
FA_5	25.00	45.60	**35.30**	38.75	46.50	**42.63**	55.50	61.50	**58.50**
FA_{10}	26.80	64.50	**45.65**	45.50	73.50	**59.50**	58.25	79.50	**64.38**
FA_{15}	26.25	64.00	**45.13**	46.60	72.60	**59.60**	56.00	78.00	**64.00**
MEAN	**25.80**	**42.52**	**39.41**	**42.09**	**56.90**	**49.50**	**55.69**	**69.88**	**62.79**
	S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)	
FA	0.34	**NS**		0.40	**NS**		0.67	**NS**	
VC	0.26	**0.54**		0.26	**1.08**		0.32	1.09	
FA x VC	0.52	**1.08**		0.50	**2.17**		0.65	2.20	

NS-Not Significant; VC_0 -Control (with out Vermicompost); VC_{10} -Vermicompost @10MT/ha; FA_0 -Control (with out Fly ash); FA_5 -Fly ash @ 5MT/ha; FA_{10} - Fly ash @10 MT/ha; FA_{15}-Fly ash @15MT/ha.

Table 2 : Effect of fly ash and vermicompost on silkworm larval duration, larval weight and effective rate of rearing (ERR%)

Fly ash (Mt/ha)	Vermicompost levels (MT/ha/yr)								
	Larval duration (days)			10 larval weight (g)			ERR (%)		
	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean
FA_0	26.80	26.50	**26.65**	30.62	42.30	**36.46**	60.55	82.50	**71.53**
FA_5	26.75	26.08	**25.92**	32.55	46.90	**39.73**	68.66	84.72	**76.69**
FA_{10}	26.08	25.42	**25.75**	35.90	50.80	**43.35**	72.50	97.62	**85.06**
FA_{15}	26.02	26.07	**26.05**	35.60	50.00	**42.80**	70.50	96.00	**83.25**
MEAN	**26.42**	**26.17**	**26.30**	**33.67**	**47.50**	**40.59**	**68.05**	**90.21**	**79.13**
	S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)	
FA	1.02	**NS**		0.22	**NS**		0.21	**NS**	
VC	0.72	**1.54**		0.23	**0.54**		0.22	0.69	
FA x VC	1.44	**3.09**		0.71	**1.09**		0.44	1.40	

NS-Not Significant; VC_0 -Control (with out Vermicompost); VC_{10} -Vermicompost @10MT/ha; FA_0 -Control (with out Fly ash); FA_5 -Fly ash @ 5MT/ha; FA_{10} - Fly ash @10 MT/ha; FA_{15}-Fly ash @15MT/ha.

higher activity of phosphatases in the soil amended with the organics could be due to more secretion of these enzymes from plant roots for solubulization and mobilization of phosphataes, which would influence the ability of plants to cope with P stressed conditions (Karthikeyan *et al.*, 2002). It is the fact that the addition of organic amendments activates microorganisms to produce extracellular enzymes (Melero *et al.*, 2006). The increase of organic matter plays an important role in protecting extra-cellular enzymes in the soil humic-mineral complex (Tabatabai, 1994).

Cellulase Activity : Cellulase activity ranged from 2.08 to 3.50, 2.03 to 2.98 and 1.04 to 2.18 mg of glucose released/mg soil/d at 30, 60 DAT and at harvest (70 days), respectively (Table 8). The treatment combination of fly ash @ 15 MT/ha/yr and vermicompost @ 10 MT/ha/yr (FA_{15} VC_{10}) has recorded about 71.3, 70.8 and 109.6% increase in cellulase activity at 30, 60 DAT and at harvest (70th day), respectively, over control (FA_0 VC_0), with out fly ash and vermicompost. The increase in cellulase activity in biologically- managed system indicates its ability in decomposition of cellulolytic materials in the soil for microbial use and improved soil health in agricultural ecosystems. Increase in cellulase activity under incubation studies due to application of fly ash was reported by Lal *et al.*, (1996).

It is concluded that addition of fly ash and organic matter (vermicompost), the quantitative (leaf yield) and qualitative characters (leaf moisture percentage and leaf moisture retention capacity) of mulberry improved significantly due to release of macro and micro nutrients in to soil from the fly ash and vermicompost. Further, after feeding the mulberry leaf

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Table 3 : Effect of fly ash and vermicompost on silkworm cocoon characters.

Fly ash (Mt/ha)	Vermicompost levels (MT/ha/yr)								
	Single cocoon weight (g)			Single shell weight (g)			Shell ratio (%)		
	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean
FA_0	1.325	1.480	**1.403**	0.126	0.245	**0.183**	09.50	16.56	**13.03**
FA_5	1.330	1.560	**1.445**	0.153	0.273	**0.210**	11.50	17.50	**14.50**
FA_{10}	1.384	1.965	**1.675**	0.222	0.406	**0.307**	16.00	20.65	**18.33**
FA_{15}	1.381	1.950	**1.666**	0.219	0.376	**0.293**	15.89	19.30	**17.60**
MEAN	**1.355**	**1.739**	**1.547**	**0.180**	**0.325**	**0.248**	**13.22**	**18.50**	**15.87**
	S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)	
FA	0.04	**N S**		0.006	**0.007**		0.72	**NS**	
VC	0.05	**0.06**		0.008	**0.008**		0.79	1.75	
FA x VC	0.11	**0.12**		0.009	**0.009**		0.93	2.60	

NS-Not Significant; VC_0 -Control (with out Vermicompost); VC_{10} -Vermicompost @10MT/ha; FA_0 -Control (with out Fly ash); FA_5 -Fly ash @ 5MT/ha; FA_{10} - Fly ash @10 MT/ha; FA_{15}-Fly ash @15 MT/ha.

Table 4 : Effect of fly ash and vermicompost on Urease activity in soil under field conditions at 30, 60 DAT and at (70th day) harvest.

Fly ash (Mt/ha)	Vermicompost levels (MT/ha/yr)								
	30 DAT µg of NH_4^+ released/g soil/h			60 DAT µg of NH_4^+ released/g soil/h			At harvest (70 days) µg of NH_4^+ released/g soil/h		
	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean
FA_0	1.78	3.46	**2.62**	1.86	3.99	**2.93**	0.89	1.95	**1.42**
FA_5	2.22	3.84	**3.03**	2.42	4.26	**3.34**	1.04	2.21	**1.63**
FA_{10}	2.90	4.46	**3.68**	3.04	4.79	**3.92**	1.45	2.51	**1.98**
FA_{15}	2.93	4.50	**3.72**	3.10	4.81	**3.96**	1.51	2.54	**2.03**
MEAN	**2.46**	**4.07**	**3.26**	**2.61**	**4.46**	**3.54**	**1.23**	**2.31**	**1.77**
	S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)	
FA	0.06	**0.14**		0.07	**0.18**		0. 05	0.10	
VC	0.05	**0.11**		0.05	**0.13**		0.04	0.09	
FA x VC	0.10	**0.24**		0.12	**0.27**		0.08	0.18	

Initial urease activity-1.25 (µg of NH_4^+ released/g soil/h); VC_0 -Control (with out Vermicompost); VC_{10} -Vermicompost @10MT/ha; FA_0 -Control (with out Fly ash); FA_5 -Fly ash @ 5MT/ha; FA_{10} - Fly ash @10 MT/ha; FA_{15}-Fly ash @15MT/ha.

produced with fly ash and vermicompost combinations the larval and cocoon characters of silkworm enhanced significantly (P<0.05) is the indication of the enhancement of leaf quality suited to the silkworm rearing. Hence, adoption of the integrated use of fly ash @ 10MT/ha/yr and vermicompost @ 10 MT/ha/yr in mulberry cultivation will bring out the quantitative and qualitative production of leaf for better silkworm cocoon crop.

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Table 5 : Effect of fly ash and vermicompost on dehydrogenase enzyme activity in soil under field conditions at 30, 60 DAT and at (70th day) harvest.

Fly ash (Mt/ha)	Vermicompost levels (MT/ha/yr)								
	30 DAT mg of TPF produced/g/d			60 DAT mg of TPF produced/g/d			At harvest (70 days) mg of TPF produced/g/d		
	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean
FA_0	5.03	5.21	**5.12**	5.02	5.28	**5.15**	2.70	2.84	**2.77**
FA_5	5.15	5.22	**5.19**	5.14	5.35	**5.25**	2.80	2.95	**2.88**
FA_{10}	5.23	5.36	**5.30**	5.25	5.47	**5.36**	2.87	3.08	**2.98**
FA_{15}	5.26	5.37	**5.32**	5.28	5.47	**5.38**	2.92	3.09	**3.00**
MEAN	**5.17**	**5.28**	**5.23**	**5.17**	**5.39**	**5.29**	**2.83**	**2.99**	**2.90**
	S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)	
FA	0.02	0.05		0.04	0.09		0.03	0.09	
VC	0.01	0.04		0.04	0.08		0.02	0.05	
FA x VC	0.03	0.06		0.08	0.16		0.04	0.11	

Initial dehydrogenase activity-2.95 mg of TPF produced/g soil/d; VC_0 -Control (with out Vermicompost); VC_{10} -Vermicompost @10MT/ha; FA_0 -Control (with out Fly ash); FA_5 -Fly ash @ 5MT/ha; FA_{10} - Fly ash @10 MT/ha; FA_{15}-Fly ash @15MT/ha.

Table 6 : Effect of fly ash and vermicompost on acid phosphatase activity in soil under field conditions at 30, 60 DAT and at (70th day) harvest.

Fly ash (Mt/ha)	Vermicompost levels (MT/ha/yr)								
	30 DAT µg of p-nitrophenol released/g soil/h			60 DAT µg of p-nitrophenol released/g soil/h			At harvest (70 days) µg of p-nitrophenol released/g soil/h		
	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean
FA_0	22.57	31.60	**27.09**	21.40	29.49	**25.45**	16.60	22.43	**19.52**
FA_5	20.67	32.01	**26.34**	20.78	30.69	**25.74**	13.91	22.89	**18.40**
FA_{10}	20.34	31.53	**25.69**	20.23	30.18	**25.21**	13.93	22.84	**18.39**
FA_{15}	20.64	31.50	**26.08**	20.68	29.98	**25.33**	13.83	22.17	**18.00**
MEAN	**21.06**	**31.54**	**26.30**	**20.77**	**30.09**	**25.43**	**14.57**	**22.69**	**18.63**
	S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)	
FA	1.02	NS		0.70	NS		0.62	NS	
VC	0.70	1.54		0.45	1.02		0.42	0.94	
FA x VC	1.42	3.08		0.90	2.06		0.85	1.87	

Initial acid phosphatase activity-10.5 µg of p-nitrophenol released/g soil/h. VC_0 -Control (with out Vermicompost); VC_{10} -Vermicompost @10MT/ha; FA_0 -Control (with out Fly ash); FA_5 -Fly ash @ 5MT/ha; FA_{10} - Fly ash @10 MT/ha; FA_{15}-Fly ash @ 15MT/ha.

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Table 7 : Effect of fly ash and vermicompost on alkaline phosphatase activity in soil under field conditions at 30, 60 DAT and at (70th day) harvest.

Fly ash (Mt/ha)	Vermicompost levels (MT/ha/yr)								
	30 DAT µg of p-nitrophenol released/g soil/h			60 DAT µg of p-nitrophenol released/g soil/h			At harvest (70 days) µg of p-nitrophenol released/g soil/h		
	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean
FA_0	20.17	27.10	**23.60**	19.34	34.69	**27.02**	12.25	17.15	**14.70**
FA_5	20.31	27.48	**23.89**	18.83	25.55	**22.19**	11.93	17.42	**14.68**
FA_{10}	19.57	27.19	**23.38**	18.15	24.79	**21.47**	11.48	16.99	**14.24**
FA_{15}	19.58	27.19	**23.39**	18.15	24.69	**21.42**	11.21	16.99	**14.60**
Mean	**19.91**	**27.24**	**23.58**	**18.62**	**27.43**	**23.03**	**11.72**	**17.14**	**14.43**
	S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)	
FA	0.35	NS		3.61	NS		0.18	0.40	
FYM	0.25	0.53		2.54	5.42		0.14	0.30	
FA x FYM	0.52	1.07		5.12	10.85		0.27	0.62	

Initial alkaline phosphatase activity-11.6 µg of p-nitrophenol released/g soil/h. VC_0 -Control (with out Vermicompost); VC_{10} - Vermicompost @10MT/ha; FA_0 -Control (with out Fly ash); FA_5 -Fly ash @ 5MT/ha; FA_{10} - Fly ash @10 MT/ha; FA_{15}-Fly ash @15MT/ha.

Table 8 : Effect of fly ash and vermicompost on cellulase activity in soil under field conditions at 30, 60 DAT and at (70th day) harvest.

Fly ash (Mt/ha)	Vermicompost levels (MT/ha/yr)								
	30 DAT mg of glucose released/ g soil/d			60 DAT mg of glucose released/ g soil/d			At harvest (70 days) mg of glucose released/ g soil/d		
	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean	VC_0	VC_{10}	Mean
FA_0	2.08	3.20	**2.64**	2.03	2.55	**2.29**	1.04	1.77	**1.60**
FA_5	2.88	2.99	**2.94**	2.18	2.68	**2.43**	1.42	1.96	**1.69**
FA_{10}	2.75	3.49	**3.12**	2.64	2.98	**2.81**	1.49	2.18	**1.84**
FA_{15}	2.75	3.50	**3.13**	2.62	2.98	**2.80**	1.46	2.18	**1.82**
MEAN	**2.62**	**3.30**	**2.92**	**2.37**	**2.80**	**2.58**	**1.35**	**2.02**	**1.74**
	S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)		S. Em ±	C.D (P≤0.05)	
FA	0.15	0.35		0.09	0.19		0.03	0.07	
FYM	0.10	0.25		0.15	0.14		0.02	0.04	
FA x FYM	0.21	0.51		0.30	0.29		0.04	0.08	

Initial cellulase activity-1.01 mg glucose released/g soil/d. VC_0 -Control (with out Vermicompost); VC_{10} -Vermicompost @10MT/ha; FA_0 -Control (with out Fly ash); FA_5 -Fly ash @ 5MT/ha; FA_{10} - Fly ash @10 MT/ha; FA_{15}-Fly ash @15MT/ha.

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Response of Green Gram (*Phaseolus aureus* Roxb.) to Integrated Application of FYM, *Rhizobium* and Vermicompost

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Experiments were carried out to study the response of green gram to the integrated application of Farm Yard Manure (FYM), Biofertilizer, *Rhizobium japonicum,* (BF) and Vermicompost (VC) under pot-culture condition. From the results, it is evident that the seedling growth, biomass production and yield of green gram were promisingly increased in all soil treatments, in general, than control. However, maximum seed germination (98.25%) was recorded in soil treated with VC alone. Soil treatment with FYM either alone or in combination with BF or/and VC increased the seedling growth of green gram at all sampling days with a maximum increase of 52.63cm/pl on 45th day sample in FYM+VC treated soil. VC+BF increased more number of root nodules (18.25/pl), leaflets (17/pl) and fresh biomass (8943mg/pl) in 45th day sample while dry biomass was maximum (2818mg/pl) in 75th day sample. Higher LAI was noted in FYM+BF treated soil on 75th day sample. The SVI was higher in soil treated with FYM+BF+VC on 75th day sample. The STI was higher in soil treated with FYM alone on 45th day sample. Maximum number of buds (14.25), flowers (14.50) and seeds (34) per plant was noted in green gram seedlings grown in soil treated with VC+BF on 75th day sample. The soil treated with FYM, BF and VC either alone or in combination favours more chlorophyll content on 45th day sampling than control as well as early (15th day sampling) and later growth periods (60th day sampling). The synthesis of protein content in green gram leaves was promoted by all soil treatments during early and later stages of growth, while it was decreased at middle growth period than control.

Keywords : Green gram, *Phaseolus aureus*, Farmyard manure, *Rhizobium*, Vermicompost.

INTRODUCTION

The use of organic manures, biofertilizers, crop residues, composts and vermicompost to have drawn the attention because they are not only economic sources of fertilizers but eco-friendly and increase the availability of nutrients to plants in soil. Though use of all the above sources in improving the growth and productivity of many crops is well documented, there are very few or insufficient work conducted on integrated application of organic manures, biofertilizers and vermicompost on green gram. Therefore, the present work was carried out to study the response of green gram, *Vigna radiata* (L) R. Wilczek. (Synonyms: *Phaseolus aureus* Roxb) var. KM-2, to the integrated application of organic manure (farmyard manure), biofertilizer (*Rhizobium japonicum*) and vermicompost on seedling growth and productivity.

MATERIAL AND METHODS

Certified seeds of green gram var. KM-2 were collected from Agricultural Department, Namakkal. Seeds treated with 0.1% mercury chloride solution and washed thoroughly with tap water and then with distilled water for several times and used. The vermicompost and biofertilizer - *Rhizobium japonicum* were obtained commercially from agrochemical stores. Farmyard manure was used as dry powder. Pot culture experiment was carried out in garden soil (sandy and loamy red soil) of Sri Paramakalyani Centre for Environmental Sciences, Department of Vermitechnology, Alwarkurichi.

Soil Treatments

T1 - Control
T2 - Farm Yard Manure (1%)
T3 - Vermicompost (1.5%)
T4 - Farm Yard Manure (1%) + Biofertilizer (*Rhizobium japonicum*) (0.5%)
T5 - Vermicompost (1.5%) + Biofertilizer (0.5%)
T6 - Vermicompost (1.5%) + Farm Yard Manure (1%)
T7 - Vermicompost (1.5%) + Farm Yard Manure (1%) + Biofertilizer (0.5 %)

Green gram (var. KM-2) plants were grown in 8" height x 10" diameter size pots filled with air-dried sand: loam soil (5Kg / pot) mixed in the ratio of 1:1. Then the pots were treated with vermicompost-VC (1.5%), farmyard manure-FYM (1%)

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and biofertilizer-BF, *Rhizobium*, (0.5%) either alone or in combination as mentioned above. The VC, FYM and BF were applied to the surface of the sandy-loam soil and mixed thoroughly. Green gram seeds (15 seeds/ pot) were sown in each pot and all the pots were watered to field capacity daily. For every treatment, four replications were maintained. The per cent seed germination and seedling growth were recorded during the growth period. The plant samples were collected at 15th, 45th and 75th days for the measurements of various morphological, biochemical and yield parameters. Twelve plants from each replication were analyzed for various parameters and the average was calculated. The various morphological parameters such as root length, shoot length, number of leaflet, leaf area index (Kalra and Dhiman, 1977), fresh and dry weights, seedling vigour index (Abdul-Baki and Anderson, 1973), number of nodules and tolerance index (Turner and Marshal, 1972); and biomolecules like chlorophyll pigments (Amon. 1949) and protein content (Lowry *et al.*, 1951) of seedlings in various treatments were recorded by using standard methods. All experimental data were averaged and analyzed statistically to find out statistical significance (Standard deviation, F-value, CD-value) by following appropriate methods such as ANOVA (Analysis of Variance - One way) method as described by Panse and Sukhatme (1985).

RESULTS

Seed germination and seedling growth : From the results, it is evident that soil treated with VC favoured maximum seed germination of 98.25% whereas it was 95% and 93.25% in soil treated with FYM + BF + VC and VC + BF, respectively. All other treatments showed a reduction in the per cent seed germination than control (Tab -1). The seedling growth (total length) of green gram was increased in all treatments as compared to control at all observations (15th, 45th and 75th day) with maximum increase of 52.63 cm/pl in soil treated with FYM+VC (T4). Soil treatments generally promoted the formation of more number of root nodules in green gram seedlings at all sampling days (except in 45th day sample of VC soil treatment). Maximum number of root nodules (18.25/ pl) was recorded in VC+BF treatment and was followed FYM+BF (13.25/ pl), FYM+VC and FYM+BF+VC (11.50/pl) in 45th day sampling (Table-1). On 75th day sampling, the number of root nodules was reduced in most of the soil treatment as compared to 15th day, 45th day sampling.

In 15th day sampling, the maximum seedling vigour index (2137) was favoured by FYM+VC+BF soil treatment, while in 45th and 75th day observations, the maximum SVI was recorded in soil treatment with VC+BF (4336) and FYM+BF+VC (4962), respectively (Table 1). The difference in tolerance index of green gram seedling towards various soil treatments was non-significant, but significant between days. All soil treatments favoured an increase of tolerance index in the 45th day sample with a maximum (2.58) in FYM treated soil, while it was less in other two sampling.

Maximum number of leaflets (17/pl) was recorded in soil treated with VC+BF at 45th day sampling, and 15th day sample (5.25/pl). At 75th day sample, a maximum of 15.75 leaflet / pl was recorded in treatments T_5 and T_7 (Table 1). All soil treatments increased the LAI than control at all sampling days (15th, 45th and 75th day) except in 15th day sample of FYM+BF+VC treatment. On 15th day sampling, FYM+VC treatment favoured maximum LAI (7.15 cm^2 / leaflet). On 45th day and 75th day observations, the soil treated with VC+BF and FYM+BF favoured maximum (19.34 and 30.45 cm^2 / leaflet) LAI, respectively.

All treatments increased the seedling fresh weight of green gram compared with control at all days of observations (Table 1). Maximum seedling fresh weight of 363, 8943 and 8.838 mg/pl was recorded in 15th, 45th and 75th day sampling of VC+BF, VC+BF and FYM+BF+VC treatments, respectively. Maximum seedling dry weight 175, 1903 and 3495 mg/pl was recorded in soil treated with VC, VC+BF and FYM+BF+VC (in 15th, 45th, and 75th day samples), respectively. Soil treated with FYM showed minimum increase in dry matter production at all sampling days as compared to control (Table 1).

Flowering and yield parameters : The number of buds developed in green gram seedlings grown in soil treated with FYM, BF and VC either alone or in combination was counted on the days of sampling and the results indicate that the increase in the number of buds in green gram seedling was significant between treatment and days while it was non-significant in treatment x days. The number of buds was more in 75th day sample, than in 45th day sampling. Maximum numbers of buds (11/pl and 14.25/pl) were recorded in the treatments with VC+BF on 45th and 75th day sampling, respectively. All soil treatments favoured an increase in the number of flowers (from 1.50/pl to 14.5/pl) in both sampling days, except a reduction (1.25/pl) in soil treated with FYM+VC at 45th day sample. Maximum number of flowers recorded in soil treated with VC+BF (3.00/pl) and VC+BF (14.50/pl), in 45th and 75th day samples, respectively (Table 2). Soil treatments significantly increased the number of pods in both sampling days with maximum pods (3.75/pl and 15.25/pl) in soil treated with VC+BF and VC, was recorded in 45th and 75th day sampling, respectively, compared to control. The pod length of green gram was increased in all soil treatments and at all sampling days than control. Maximum increase of pod length (6.50 cm/pod) was recorded in soil treated with FYM+BF+VC on 45th day sample over control; while it was minimum increase of pod length (3.73 cm/pod) on 75th day sampling in FYM treated soil. Maximum number of seed (34/pl) was recorded on 75th day sampling, in soil treated with VC+BF, while on 45th, maximum number of seeds was produced by VC (16/pl).

Bio-molecule content : The chlorophyll-a content decreased in 15 day old green gram seedlings grown in all soil treatments, while it was increased 45th in day samples compared to control. In 75th day samples, the chlorophyll-a increased in most of the soil treatments with a maximum of 215µg/g fr wt in soil treated with VC alone (T3), except in treatments T2 and T7 which showed a reduction as compared to control (Table-2). Maximum chlorophyll-b content (157 µg/g fr. wt.) was recorded in soil treated with FYM in 45th day sampling, while in 15th and 75th day samples, it was 96 and 104 µg/g fr. wt. in treatments T3

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and T6, respectively. In FYM treated soil, the chlorophyll -b content was reduced to 33 µg/g fr. wt (in 15th day sample) as compared to control. All treatments promoted the total chlorophyll content at 45th day observations as compared to control, while it was reduced in 75th day samples. On 15th day, maximum total chlorophyll content (214 µg/g fr. wt) was recorded by VC soil treatment while all other treatments reduced the chlorophyll pigments. In 45th and 75th day observation, the maximum chlorophyll content was noted in soil treated with FYM+VC (280 µg/g fr. wt) and VC+BF (310 µg/g fr. wt), respectively (Table 2).

The maximum protein content (12.06 mg/g fr. wt.) was favoured by FYM+BF+VC soil treatment in 15th day sample. Similarly on 45th and 75th day observations, the maximum protein content (44.43 mg/g fr. wt) and (47.58 mg/g fr. wt.) was recorded in soil treated with VC respectively. However, FYM, FYM+BF and FYM+BF+VC treatment reduced the protein content on 45th day sampling than control (Table 2).

DISCUSSION

Seed germination and seedling growth are vital for continuation of plant life. Seeds and seedlings are extremely vulnerable to environmental condition, which is very important for initiating and triggering the intricate sequence of metabolism essential for germination and growth of seedlings. The results of biofertilizer treatments on green gram indicate that the soil treatments such as FYM, FYM+VC and FYM+BF significantly suppressed the seed germination of green gram under pot culture conditions compared to control (Table 1), whereas the VC soil treatment either alone or in combination with FYM or/ and BF promoted the same. The increase in germination percentage at certain soil treatments indicates the stimulation by the treatment (Biradar *et al.*, 1989). Gupta (1979) reported the inhibition of seed germination of pulses because of chlorides and other dissolved solids present in the irrigation water. Rajannan and Kandasamy (1990) reported that the reduction in the crop growth of rice, green gram, maize, cowpea, finger millet might be attributed to cumulative effect of acidic pH, high amounts of dissolved solids such as chlorides which could create osmotic imbalance on the root system. The promotory effect of soil treatments with FYM, BF and VC either alone or in combination on seedling growth parameters of green gram recorded in the present study were on confirmative by many workers. This may be due to the application of FYM, BF and VC which improves physico-chemical as well as biological properties of soil as suggested by Ismail (1997).

Soil treatments in the present study, generally, increased the number of root nodules in green gram seedlings at all sampling days tested as reported by Reena and Bagyaraj (1990). According to them, increase in nodulation can be due to increased supply of phosphorus to the host plant or due to the production of plant hormones by the organisms.

The number of leaflets in green gram seedlings was increased in most of the soil treatments at all sampling days than control. The LAI was also increased in most of the soil treatment. Earthworm-enhanced plant growth by improving the physical and chemical condition of soil as well as by producing hormones in vermicompost (Nielson, 1951; Nijhawan and Kanwar, 1952). They observed that the application of earthworm compost increased the plant height, number of leaves, etc. All soil treatments promoted the biomass production (seedling fresh and dry weight) of green gram seedlings than control. The increase in seedling biomass production may be strongly correlated with increased accumulation of nitrogen due to *Rhizobium* sp. As reported by Jamaluddin *et al.* (1995). Dual inoculation favouring the biomass production on various crop was documented earlier (Leopard and Hopman, 1991; Sekar *et al.*, 1995).

The number of buds and flowers produced in green gram seedlings was generally increased by most of the soil treatments than control. However, all soil treatments improved the yield parameters (number of pods, seeds and pod length) of green gram at all sampling than control. Similar observations were also made by Tomiti *et al.* (1983), Ismail (1997) and Das *et al.* (2002).

The response of green gram seedling growth in soil treated with FYM, BF and VC either alone or in combination was highly positive. Because, the seedling growth, biomass production and yield were promisingly increased in all soil treatments than control. However, the result of present investigation reveals that vermicompost soil treatment along with biofertilizer treatment favours maximum increase of seedling growth, biomass production, etc., than all other treatments tested.

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Table 1 : Effect of integrated Farmyard manure (FYM), Biofertilizer (BF) and Vermicompost (VC) application on the seedling growth of green gram var. KM2.

Soil treatments	Age of plant (Days)	SG# (%)	Seedling (length) growth (cm / pl)	No of root nodules	No of leaflet / pl	LAI (cm^2/leaflet)	Seedling fresh weight (mg / pl)	Seedling dry weight (mg / pl)	Seedling vigour index (SVI)	Seedling tolerance index (TI)
T-1. Control	15	91.50 ± 7.23	18.15 ± 2.53	4.50 ± 2.87	2.50 ± 0.50	5.34 ± 1.39	260 ± 148	115 ± 36	1672 ± 314	1.00 ± 0.00
	45		28.18 ± 9.14	7.25 ± 0.83	13.50 ± 1.50	15.01 ± 2.91	3933 ± 730	555 ± 260	2614 ± 979	1.00 ± 0.00
	75		39.78 ± 2.02	5.25 ± 2.59	13.50 ± 2.50	18.41 ± 2.75	4473 ± 1550	1320 ± 460	3641 ± 91	1.00 ± 0.00
T-2. FYM	15	81.75 ± 3.03	18.98 ± 1.46	7.25 ± 2.59	2.50 ± 1.30	5.52 ± 0.36	280 ± 23	135 ± 18	1550 ± 108	1.28 ± 0.11
	45		34.53 ± 3.41	7.50 ± 4.15	11.75 ± 2.50	15.66 ± 2.73	4078 ± 750	625 ± 130	2820 ± 28	2.58 ± 2.15
	75		46.58 ± 6.48	6.00 ± 2.92	14.75 ± 3.27	23.38 ± 1.27	4835 ± 2400	1408 ± 650	3794 ± 448	1.17 ± 0.18
T-3. VC	15	98.25±3.03	19.45 ± 1.63	8.25 ± 4.15	5.00 ± 0.00	6.65 ± 1.57	345 ± 63	175 ± 34	1910 ± 156	1.42 ± 0.14
	45		40.00 ± 3.74	6.50 ± 3.20	15.50 ± 1.50	18.88 ± 3.55	5935 ± 1620	1360 ± 230	3931 ± 51	2.31 ± 1.59
	75		42.95 ± 3.66	7.00 ± 3.32	14.75 ± 1.79	19.54 ± 5.85	5318 ± 1300	2695 ± 620	4217 ± 352	1.02 ± 0.21
T-4. FYM +VC	15	88.25 ±5.36	21.30 ± 4.46	9.00 ± 3.67	3.75 ± 1.30	7.15 ± 0.38	343 ± 70	163 ± 32	1895 ± 212	1.14 ± 0.03
	45		38.90 ± 2.81	11.50 ± 5.94	14.00 ± 0.00	18.08 ± 4.33	5185 ± 1530	1020 ± 280	3425 ± 164	2.47 ± 2.26
	75		52.63 ± 3.70	7.50 ± 3.04	14.50 ± 3.84	21.23 ± 6.10	6103 ± 780	2183 ± 830	4660 ± 590	1.33 ± 0.19
T-5. FYM+BF	15	83.50±3.50	19.30 ± 2.18	10.00 ± 1.58	4.25 ± 1.30	6.01 ± 0.89	333 ± 58	143 ± 36	1613 ± 202	1.37 ± 0.17
	45		38.88 ± 2.20	13.25 ± 3.10	10.25 ± 1.50	18.91 ± 1.91	5238 ± 2620	998 ± 420	3249 ± 266	2.53 ± 2.50
	75		51.88 ± 4.26	8.50 ± 2.87	15.75 ± 3.42	30.45 ± 7.35	5978 ± 2000	1538 ± 740	4324 ± 491	1.24 ± 0.26
T-6. VC+BF	15	93.25±4.60	21.35 ± 4.02	8.75 ± 2.68	5.25 ± 1.09	6.43 ± 2.40	363 ± 22	150 ± 25	1993 ± 398	1.41 ± 0.21
	45		46.30 ± 9.77	18.25 ±2.59	17.00 ± 2.10	19.34 ± 1.08	8943 ± 3150	1903 ± 650	4330 ± 969	2.49 ± 1.62
	75		49.19 ± 7.47	8.25 ± 2 .86	15.00 ± 2.12	24.12 ± 3.28	7050 ± 2320	2818 ± 1290	4586 ± 729	1.17 ± 0.34
T-7. FYM+BF+VC	15	95.00 ± 5.43	22.28 ± 4.44	10.50 ± 1.80	2.75 ± 1.30	4.21 ± 1.35	260 ± 82	140 ± 23	2137 ± 289	1.29 ± 0.36
	45		39.53 ± 3.00	11.50 ± 5.94	16.25 ± 1.30	17.42 ± 4.16	6450 ± 350	1333 ± 90	3751 ± 465	2.35 ± 1.72
	75		52.40 ± 10..9	6.50 ± 4.15	15.75 ± 2.77	24.47 ± 2.40	8838 ± 4940	3495 ± 1760	4962 ± 1000	1.19 ± 0.19

CD (P = 0.05)	CD / F	CD	F	CD	F	CD	F	CD	F	CD	F	CD	F	CD	F	CD	F
F-value: Between Treatment	8.18 /*	4.86	**	2.91	**	1.92	*	3.12	*	1.68	*	0.56	**	472	**	0.83	NS
Between Days	---	3.18	**	1.90	**	1.26	**	2.04	**	1.10	**	0.36	**	309	**	0.55	**
Treatment x Days	---	8.41	NS	5.04	NS	3.33	NS	5.40	NS	2.90	NS	0.97	NS	817	NS	1.44	NS

= Per cent seed germination calculated on 10th DAS; ± = Standard Deviation (SD); * & ** Significance at 1% and 5% level respectively; NS=Non-significance.

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Table 2 : Effect of integrated Farmyard manure (FYM), Biofertilizer (BF) and Vermicompost (VC) application on yield parameters and biomolecule content in green gram var. KM2 seedling.

Treatments	Age of plant (Days)	Number of buds/plant	Number of flowers/ plant	Number of pods/plant	Pod length (cm / pod)	Number of seeds/plant	Chlorophyll content (µg/g fr. wt.)			Protein content (mg/g fr.wt.)
							Chl.-a	Chl.-b	Total Chl.	
T-1. Control	15	-----	-----	-----	-----	-----	119 ± 70	78 ± 63	197 ± 13	10.92 ± 2.43
	45	6.00 ± 3.03	1.50 ± 0.50	1.25 ± 0.43	2.88 ± 1.52	2.50 ± 0.87	94 ± 11	130 ± 32	224 ± 22	34.76 ± 3.65
	75	6.25 ± 1.09	8.75 ± 3.96	4.00 ± 1.58	3.25 ± 0.55	17.25 ± 1.92	189 ± 44	123 ± 48	312 ± 12	27.48 ± 5.02
T-2. FYM	15	-----	-----	-----	-----	-----	49 ± 27	33 ± 4	82 ± 28	10.97 ± 0.50
	45	6.25 ± 3.03	1.75 ± 1.30	1.50 ± 0.50	3.55 ± 2.18	4.50 ± 1.12	114 ± 7	157 ± 16	271 ± 22	25.98 ± 8.35
	75	7.75 ± 4.26	10.75 ± 1.48	7.25 ± 2.38	3.73 ± 0.97	18.75 ± 2.59	181 ± 59	74 ± 14	255 ± 50	27.68 ± 8.60
T-3. VC	15	-----	-----	-----	-----	-----	118 ± 69	96 ± 47	214 ± 105	11.37 ± 1.16
	45	9.25 ± 1.92	1.50 ± 0.50	2.75 ± 1.48	5.78 ± 0.98	16.00 ± 5.52	136 ± 49	136 ± 28	272 ± 27	44.43 ± 5.06
	75	13.00 ± 2.24	13.75 ± 3.78	15.25 ± 0.83	4.50 ± 1.21	27.00 ± 2.29	215 ± 10	88 ± 43	303 ± 48	47.58 ± 5.12
T-4. FYM + VC	15	-----	-----	-----	-----	-----	48 ± 27	44 ± 15	92 ± 41	11.76 ± 2.06
	45	8.25 ± 1.48	1.25 ± 0.43	2.50 ± 0.50	5.08 ± 1.29	5.75 ± 2.28	167 ± 66	113 ± 36	280 ± 26	35.38 ± 6.33
	75	8.50 ± 1.12	14.00 ± 2.55	8.75 ± 3.77	5.68 ± 0.64	22.75 ± 3.83	205 ± 20	87 ± 39	292 ± 19	38.11 ± 5.64
T-5. FYM + BF	15	-----	-----	-----	-----	-----	60 ± 16	37 ± 14	97 ± 12	11.95 ± 2.46
	45	8.25 ± 4.02	1.75 ± 0.80	2.00 ± 0.71	3.65 ± 1.73	5.75 ± 2.86	109 ± 9	146 ± 22	255 ± 29	29.41 ± 2.20
	75	9.75 ± 4.26	10.25 ± 2.49	6.25 ± 3.49	4.70 ± 1.48	20.50 ± 6.18	208 ± 10	56 ± 17	264 ± 25	32.90 ± 3.32
T-6. VC + BF	15	-----	-----	-----	-----	-----	79 ± 34	61 ± 31	140 ± 58	11.69 ± 1.28
	45	11.00 ± 3.67	3.00 ± 0.71	3.75 ± 1.48	5.85 ± 1.35	10.75 ± 0.83	157 ± 51	111 ± 51	268 ± 19	36.20 ± 3.23
	75	14.25 ± 3.49	14.50 ± 2.96	7.25 ± 1.92	6.43 ± 0.83	34.00 ± 5.24	206 ± 20	104 ± 28	310 ± 26	38.49 ± 3.28
T-7. FYM+BF+VC	15	-----	-----	-----	-----	-----	67 ± 14	60 ± 46	127 ± 56	12.06 ± 1.49
	45	7.75 ± 1.48	2.25 ± 1.64	3.25 ± 0.43	6.50 ± 0.73	14.50 ± 2.69	110 ± 12	142 ± 21	252 ± 27	27.05 ± 8.13
	75	11.50 ± 4.03	11.00 ± 1.22	6.50 ± 2.60	5.93 ± 1.45	28.25 ± 6.22	173 ± 40	78 ± 34	251 ± 25	33.41 ± 2.95

CD (P = 0.05)	CD	F	CD	F	CD	F	CD	F	CD	F	CD	F	CD	F	CD	F	CD	F
F-value: Between Treatment	3.46	**	2.36	NS	2.39	**	1.50	**	4.54	**	0.035	NS	0.033	*	0.047	NS	4.30	**
Between Days	1.85	*	1.26	**	1.28	**	0.80	NS	2.43	**	0.023	**	0.022	*	0.031	**	2.82	**
Treatment x Days	4.90	NS	3.33	NS	3.38	**	2.11	NS	6.42	NS	0.061	NS	0.058	NS	0.081	NS	7.45	**

± = Standard Deviation (SD); * & ** Significance at 1% and 5% level respectively; NS=Non-significance

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Effect of Season and Drupe Size on the Chlorophyll Content and Chlorophyll Stability Index of the Teak Seedlings (*Tectona grandis* Linn. F)

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Physiological parameters like chlorophyll content and chlorophyll stability index explicates the physiological activities in plants and thereby influence the performance of seedlings. Hence a study was undertaken to find out the influence of season and drupe size on the physiological parameters of teak seedlings as evidenced through the chlorophyll content and chlorophyll stability index. Teak fruits collected from Nilambur Forest Division of Kerala State were size graded into diameter classes 9 - 12 mm, 12 - 15 mm and 15 - 18 mm. After pre-treatment they were sown in standard nursery bed and the germinants belonging to different size grades were polypotted. Chlorophyll content and chlorophyll stability index of the size graded seedlings were determined in rainy (June-September), winter (October-January) and summer (February-May) season. The results showed that season significantly influenced the chlorophyll content and chlorophyll stability index of the seedlings with higher values during winter. With regard to drupe size, the seedlings belonging to 15-18 mm grade recorded higher chlorophyll content.

Keywords : *Tectona grandis*, Drupe size, Chlorophyll content, Chlorophyll stability index.

INTRODUCTION

Photosynthesis is the key process that converts light energy to forms of energy that can be utilized by the living organisms. Chlorophyll a and b pigments are associated with light harvesting process at the antenna. Seasonal variations of temperature, rainfall, humidity and wind may exert a pronounced control over the physiological processes in plants. Physiological parameters like chlorophyll content and chlorophyll stability index influence the performance of seedlings. Seed size also is a crucial plant trait that may potentially affect not only immediate survival of the plant but also the subsequent development of it. A close relationship between drupe size and germination and subsequent plant development of teak has been concluded by several workers (Latif, 1982; Viswanath *et al.*, 1995; Manonmani and Vanangamudi, 2003). Whereas, Jayasankar *et al.*, (1999) found that the drupe size characteristics had no or weak correlation with germination percentage of teak drupes. Indira *et al.* (2000) also reported that drupe size did not have any influence on seedling survival and growth. Literature on the influence of season and drupe size on the physiological parameters of teak seedlings is scanty. Therefore, a study was undertaken to find the influence of season and drupe size on the physiological parameters of teak seedlings as evidenced through the chlorophyll content and chlorophyll stability index.

MATERIAL AND METHODS

The study was conducted at the College of Forestry, Kerala Agricultural University, Thrissur, Kerala during June, 2002 to June, 2003. The nursery area is located at 40 meters above mean sea level at 10°32'N latitude and 76°26'E longitude. The area experiences a warm and humid climate with distinct rainy season. The drupes were obtained during January - February of 2002 from the Cherupuzha plantation located in Karulai Range, Nilambur Forest Division of Kerala and established in 1971. The drupes were graded using the sieves of diameter 9, 12 and 15 mm and designated as 9-12 mm (small), 12-15 mm (medium) and 15-18 mm (large) classes respectively. As the drupes below 9 mm were inferior in germination percentage and the drupes above 18 mm diameter were too few in number (Jijeesh and Sudhakara, 2007) they were discarded from the study. The drupes were pre-treated by termites to remove the mesocarp (Chacko, 1998) and sown in standard nursery beds. The germinated seedlings belonging to different size grades were planted in polythene bags (gauge 300) of size 17" x 17", filled with sand, soil and farmyard manure in the ratio of 1:1:1. Eight seedlings from each size grade were randomly selected at monthly intervals for physiological observations during (June-Septermber), winter (October-January) and summer (February-May) seasons. The tissue from the leaves of second node from the tip was used to determine the chlorophyll content and chlorophyll stability index

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of the seedlings. The average weather parameters during different seasons are presented in Table 1.

The chlorophyll content of the seedlings was estimated as suggested by Schoaf and Livum (1976). 0.1g leaf sample was taken in a test tube and 10 ml of Dimethyl Sulfoxide (DMSO) was added and kept overnight. The absorbance of solution was read using Spectronic-20 spectrophotometer at wavelengths of 645, 652 and 663 nm against solvent blank. the chlorophyll content was calculated using the formulae :

$$\text{Chlorophyll } a \text{ (mg/g tissue)} = \frac{12.7\,A_{(663)} - 2.6A_{(645)} \times V}{1000 \times W}$$

$$\text{Chlorophyll } b \text{ (mg/g tissue)} = \frac{22.9\,A_{(645)} - 4.68\,A_{(663)} \times V}{1000 \times W}$$

$$\text{Total chlorophyll (mg/g tissue)} = \frac{20.2\,A_{(645)} + 8.62\,A_{(663)} \times V}{1000 \times W}$$

$$= \frac{A_{(652)} \times 1000 \times V}{34.5 \times 1000 \times W}$$

Where, Ai = absorbance at wavelength i, V = final volume of chlorophyll extract and W = fresh weight of tissue extract.

The chlorophyll stability index of the seedlings was estimated as suggested by Murthy and Majumdar (1972). Leaf samples (0.1 g) were taken in two test tubes and 50 ml of distilled water was added to each. One tube was subjected to heat in water bath at 55°C ± 1°C for exactly 30 minutes. The other tube served as control. The leaf tissue was removed and blotted gently. 10 ml of DMSO was added to leaf tissue and kept overnight. The absorbance of this extract was recorded at a wavelength of 652 nm using spectronic-20 colorimeter. The difference in two readings was used to calculate the Chlorophyll stability index.

$$\text{Chlorophyll content} = \frac{A_{(652)} \times 1000 \times V}{34.5 \times 1000 \times W}$$

$$\text{Chlorophyll stability index} = \frac{\text{Control - treated} \times 100}{\text{Control}}$$

The experiment was conducted as repeated measurements in completely randomised design over different seasons. The ANOVA was conducted for repeated measurements over different seasons and the treatment means were compared with lsd (least significant difference) wherever necessary.

RESULTS

Individual effect of season and drupe size : All the physiological parameters of the teak seedlings varied significantly (p=0.01) due to season (Table 2). Average chlorophyll a (2.14 mg/g tissue), total chlorophyll (4.26 mg/g tissue) and CSI (23.84) were the highest during season whereas highest chlorophyll b content (2.08 mg/g tissue) was observed in summer season which was at par with that in winter. On an average, physiological parameters were the lowest during rainy season.

The effect of drupe size on chlorophyll content (a, b and total) also was significant (p = 0.01). CSI does not vary significantly due to drupe size. Average chlorophyll a (2.12 mg /g tissue), chlorophyll b (2.00 mg/g tissue) and total chlorophyll were significantly higher in seedlings belonging to 15 - 18 mm grade. Meanwhile, the seedlings belonging to 9-12 mm drupe grade recorded the lowest value.

Interaction effects of season and drupe size : The interaction effects of season and drupe size on chlorophyll a (p=0.01), chlorophyll b and total chlorophyll (p=0.05) also were significant (Table 3). The highest chlorophyll a (2.32 mg/g tissue), chlorophyll b (2.14 mg/g tissue) and total chlorophyll (4.44 mg/g tissue) were observed in the seedlings belonging to 15-18 mm during winter season. The lowest values for the same were recorded for 9-12 mm seedlings rainy season.

DISCUSSION

Chlorophyll content of leaves is a useful indicator of both potential photosynthetic productivity and general plant vigour (Alonso *et al.* 2002; Zarco-Tejada *et al.* 2002). In addition, changes in the amount of chlorophyll may be a part of adaptive responses (Morales *et al.* 2002). Our experimental results indicate that chlorophyll content both chlorophyll a and chlorophyll b and therefore the total chlorophyll are significantly higher in the winter season and it was lowered in summer season. In rainy season, chlorophyll b and total chlorophyll content was slightly decreased when compared with summer. Chowdhury and Johri (2003) also reported higher chlorophyll content in the winter season. Chlorophyll stability index (CSI) is an indication of the stress tolerance capacity of plants and it indicates how well chlorophyll can perform under stress. In our study the CSI was significantly higher during winter season. Koleyorease, (1958) stated that a higher CSI helps plants to withstand stress through better availability of chlorophyll that leads to increase in photosynthetic rate, more dry matter production, and higher productivity.

The variation in number of teak drupes and physical characteristics of the drupes belonging to five size grades of Nilambur Forest Division was described by Jijeesh and Sudhakara (2007). Sudhakara and Jijeesh, (2008) reported the positive correlation of drupe size obtained with germination parameters. A close relationship between drupe size and germination and subsequent plant development of teak has been observed by several workers (Banick, 1978; Kumar, 1979;

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Viswanath *et al.*, 1995; Manonmani and Vanangamudi, 2003). In the present study, the chlorophyll a chlorophyll b and thereby the total chlorophyll was significantly higher in seedlings obtained from 15-18 mm size grade. This higher growth of seedlings obtained from large drupes may be attributed to the increased chlorophyll content of their leaves. In contrary Sudhakara *et al.*, (1995) has reported that the seed size did not influence the chlorophyll content of the *Ceiba pentandra* seedlings.

Leaf chlorophyll content provides valuable information about physiological status of plants and there by the biomass production of the plants. In summary, the environmental conditions in winter season favoured the higher production of chlorophyll. The seedlings from large seed size grades possessed higher chlorophyll content. Hence, the effect of drupe size variations in growth performance of teak seedlings can be attributed to the higher chlorophyll content and its stability. There is a potential for further physiological processes to explain the better growth performance of the seedlings obtained from large sized drupes in teak.

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Table 1 : The weather parameters of the study area during June, 2002 to June, 2003.

Season	Month	Temperature (°C)			Relative humidity (%)		Rainfall	
		Max.	Min.	Mean	Morning	Evening	Rainfall (mm)	Rainy days
Rainy	June	31.8	31	31.4	93	78	533.5	22
	July	29.8	21.4	25.6	94	74	354.2	21
	August	31.4	21	26.2	94	78	506.6	19
	September	33.3	21.6	27.3	92	62	124.0	8
Winter	October	33.4	22.5	27.8	91	74	387.7	19
	November	33.2	22.3	27.8	83	60	22.1	3
	December	33.4	16.8	25.1	72	45	0	0
	January	35.3	19.5	27.4	66	34	0	0
Summer	February	36.8	21.6	29.2	83	43	162.1	5
	March	36	20.4	28.2	86	37	94.8	4
	April	37.8	23.4	30.6	86	58	23.8	3
	May	35.6	23.5	29.5	88	56	40.3	3

Table 2 : Individual effects of season and drupe size on the physiological parameters of the teak seedlings.

Factor	Physiological parameters	Chlorophyll a (mg/g tissue)	Chlorophyll b (mg/g tissue)	Total Chlorophyll (mg/g tissue)	CSI
Season	Rainy	1.836^{b}	1.470^{b}	3.338^{c}	20.643^{b}
	Winter	2.140^{a}	2.014^{a}	4.259^{a}	23.854^{a}
	Summer	1.798^{b}	2.079^{a}	3.856^{b}	20.204^{b}
Drupe size	Large	2.122^{a}	2.003^{a}	4.032^{a}	21.686
	Medium	1.857^{b}	1.841^{b}	3.795^{b}	21.622
	Small	1.796^{b}	1.719^{b}	3.626^{b}	21.394

The values with same superscript with in a column do not vary significantly.

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Table 3 : The variation in physiological characters of the teak seedlings as influnced by season and seed size.

Season	Size (mm)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Chlorophyll stability index
Rainy	15 - 18	2.19^{ab}	1.75^{b}	3.73^{b}	19.77
	12 - 18	1.76^{c}	1.55^{c}	3.49^{c}	20.81
	9 - 12	1.56^{f}	1.31^{c}	3.04^{c}	20.27
Winter	15 - 18	2.32^{a}	2.14^{a}	4.44^{a}	23.40
	12 - 15	2.10^{bc}	2.13^{a}	4.42^{a}	24.55
	9 - 12	1.99^{cd}	1.77^{b}	3.91^{b}	24.26
Summer	15 - 18	1.86^{bc}	2.13^{a}	3.93^{c}	22.10
	12 - 15	1.72^{ef}	2.01^{a}	3.73^{b}	18.37
	9 - 12	1.81^{e}	2.06^{a}	3.93^{b}	19.92

The values with same superscript with in a column do not vary significantly.

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Nutrient Transfer in Mangrove Habitat with Reference to *Avicennia marina* (Forsk.) Vierh.

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The mangrove ecosystem is a fragile complex and dynamic ecosystem, which is dependent on a number of environmental factors. Mangroves like any other plant need their macro and micronutrients, which they obtain from the medium in which they grow. Very often the nutrients in the growth medium many become inaccessible to the plants due to unfavorable factors like pollution, acidity, alkalinity etc.

The present investigation was undertaken to study the effect of pollution on the nutrient transfer in *Avicennia marina,* an intertidal, salt excreting sps. of the mangroves. Soil and Plants from the two sampling sites *viz.* Eastern Express Highway (between Bhandup - Vikhroli) and Palghar were collected in the month of September 2005 and analyzed for various nutrients *viz.* Nitrogen, Phosphorus, Calcium, Magnesium, Sodium, Potassium and Sulphur.

In general, the concentration of all the nutrients except sulphur was maximum in the soil followed by leaves and minimum was in stems; however sulphur showed a reverse trend, being more in the plant body than in the soil.

The results also show the Palghar site, which happens to be comparatively free from vehicular pollution, to be more nutrient rich than eastern express highway site.

Keywords : Mangroves, Nutrient transfer, *Avicennia marina.*

INTRODUCTION

Mangroves are tropical and subtropical littoral plants growing at the interface between the land and the sea. They need their macro and micronutrients to live in like any other plant and they obtain their nutrients from the medium in which they grow. Very often the nutrients in the growth medium may become inaccessible due to unfavorable factors like pollution, acidity, alkalinity etc. Some of the important minerals required for plant growth are nitrogen, phosphorus, potassium, calcium, magnesium, sodium and sulphur.

In view of the importance of the minerals to the plants and also to the members of the fragile mangrove ecosystem, the present study was undertaken. *Avicennia marina* (grey mangroves) a salt and pollution tolerant species, easily available in the intertidal zone was selected for the purpose of investigation as the representative member of the mangrove ecosystem.

The plant material (leaf, stem) and soil was analyzed for the nutrients like Nitrogen, Calcium, Phosphorus, Magnesium, Sodium, Potassium and Sulphur.

MATERIAL AND METHODS

Two sampling sites were selected for the purpose. The first site was the Eastern Express Highway Site (EEHS) (between Bhandup - Vikhroli) and the other was palghar site (PS). The plant material and soil was collected from these two sites in the month of September 2005.

Ashing and digestion of plant material was done by dry ashing method. Different methods were used for the estimation of various nutrients. Nitrogen was estimated by kjeladhl method (Trivedi and Goel 1987), Phosphorus by stannous chloride method, Calcium and Magnesium by EDTA titrimetric method, (APHA 1975) Sodium and Potassium by flame photometric method while turbidimetric method was used for the purpose of sulphur estimation.

RESULTS AND DISCUSSION

The overall results obtained for the various nutrients in the present investigation are depicted in the Table 1. The general trend for various minerals except sulphur was maximum concentration in the soil followed by leaves and minimum in the stems. However sulphur followed a reverse trend being more in the plant body than in the soil. The results also indicate the PS of be more nutrient rich when compared

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with the EEHS; however sulphur concentration was more at EEHS than at PS.

Concentration of Nitrogen at PS was 260mg/100g in soil, 160mg in leaves and 40mg/100g in the stem, whereas at EEHS it was 150, 41 and 10mg/100, in soil, leaves and stem respectively.

Availability of nitrogen along with other minerals is important in productivity of marine and coastal ecosystem. Lack of nitrogen in particular leads to limitation of growth and productivity in Mangroves. (Botto and Wellington 1983). The nitrogen status of the plants especially of the leaves not only determines the contribution of mangroves to offshore nutrient cycling but also to internal nutrient cycling of mangrove forests (Rao 1994).

Nitrogen is transported from the soil through the stem to the leaves; hence stem accounts for lesser concentration of the nutrients when compared with the leaves. (Jane 1996). Higher nitrogen content of the leaves in the present investigation is confirmed by the findings of Joshi G.V., 1975 and Joshi G.G., 1962 for mangroves in general and Kotmire *et al.* (1979) for *A. officinalis* in particular. Higher nitrogen concentration of soil followed by leaves and stem are also in keeping with the studies of Chih-Yu-Chiun *et al.* 1996, who worked with mangrove soils incorporated with 15N labeled ammonium sulphate.

Phosphorous concentration at PS ranged from 1400mg/100g in soil to 98 mg in leaves and 76mg/100g in stem and it was 740, 38 and 24 mg/100g respectively at EEHS. Phosphorus is an important nutrient required by the plants, its deficiency in the soil can prove to be a limiting factor in the primary productivity of the plants. (Iika *et. al.,* 1999). Higher concentrations of phosphorus in the leaves of salt excerting species like *A. marina* was also demonstrated by Subramanian and Venugopalan (1983). Higher concentrations of phosphorus in the leaves in comparison to the stem were also reported by Alongi *et al.* (1992) for *A. marina* from Western Port Bay, Australia and by Shah (1989) for *A. marina* from Sunderbans.

There was a major difference in the calcium concentration of (soil and plant) material collected from PS and EEHS and it was also the highest of all the nutrients in the present investigation. The calcium values were 2965.9mg/100g in soil at PS and 1763.5mg/100g at EEHS. Leaves showed 2725.4mg and 801.6mg/100g of calcium respectively at these two sites the values in the stem being 1603.2mg and 480.9mg/100g respectively. Very high concentrations of calcium, similar to the present investigation were also reported by Joshi (1975), for *A.alba* and *A. officinalis*.

Magnesium was the second highest mineral at PS (2485 mg/100g in soil, 2241.5 mg in leaves and 1656mg/100g in stem) and third highest at EEHS. Concentration at EEHS ranged from 974.5mg in soil, 828.4mg in leaves and 731mg/100g in stem. Joshi (1975) also reported lower values of Magnesium in comparison to calcium for *A. marina* and other mangroves.

1200mg/100g of Sodium was recorded for soil at EEHS; it was 710mg for leaves and 310mg/100g for stem. The corresponding values at PS were 1890mg, 1250mg and 680mg/100g. Sodium is one of the important nutrient of halophytes, which helps in maintaining the higher levels of osmotic pressure required by these plants, (Macnae 1968). *A marian* is a salt tolerant species and hence shows higher values of sodium and potassium. (Kotmire 1979), especially in the leaves (Kulkarni and Bhosale 1983).

There was not much difference in potassium values of the samples from these two sites, but still it was less at EEHS and more at PS. Sodium levels in the plants help to maintain the values of potassium, which in turn helps in salt tolerane by the plant (Kotmire, 1979), thus Na/K ratio is of utmost importance in deciding the salt tolerating capacity of the mangroves (Joshi 1975).

Concentration of sulphur showed a reversal of the order, being less in the soil and more in the plant body and also being more at EEHS and less at PS. The values for PS were 0.0370mg/100 in soil. 3mg in stem and 5mg/100g in leaves. At EEHS they were 0.1303 mg in soil, 20mg in stem and 40mg/100g in leaves. Higher concentrations of sulphur at EEHS are probably due to higher levels of vehicular pollutions at this site. More quantity of sulphur in the plant body points towards the tendency of the plant to accumulate higher concentration of the nutrient due to its lesser availability.

Based on the results of the investigation it can be concluded that, the marked difference in the nutrient levels of PS and EEHS could be due to the level of pollution at EEHS. PS is nutrient rich probably because the site is free from vehicular exhaust and other kinds of pollution whereas EEHS is constantly being exposed to it and hence has lower nutrient contents.

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Table 1 : Concentration of various nutrients (mg./100g) in the soil sample and plant material collected from the selected sites.

Nutrient	Site	Leaf	Stem	Soil
Nitrogen	Eastern express highway	41	10	150
	Palghar	160	40	260
Phosphorus	Eastern express highway	38	24	740
	Palghar	98	76	1400
Calcium	Eastern express highway	801.6	480.9	1763.5
	Palghar	2725.4	1603.2	2965.9
Magnesium	Eastern express highway	828.4	730.9	974.5
	Palghar	2241.5	1656.8	2485.2
Sodium	Eastern express highway	710	310	1200
	Palghar	1250	680	1890
Potassium	Eastern express highway	660	300	800
	Palghar	800	350	870
Sulphur	Eastern express highway	40	20	0.1303
	Palghar	5	3	0.0370

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Response of Irrigated Castor Hybrid (*Ricinus communis* L.) to Magnesium Nutrition

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Field trials were conducted to study the response of magnesium nutrition to castor hybrid TMVCHI (*Ricinus communis* L.) under irrigated condition in *Typic Rhodustalf* of Tamil Nadu, India during 2006-08. The eleven treatments includes NPK - Recommended Dose of NPK fertilizers (60:30:30 Kg NPK/ha) alone and with $MgCl_2/MgSO_4$ @ 25, 50 & 75 Kg/ha as soil application and as 0.5% / 0.1% spray on 30, 60 & 90 DAS which were replicated thrice in RBD. The results revealed that the soil application of $MgSO_4$@ 25Kg/ha recorded higher castor seed yield of 4165 Kg/ha followed by $MgCl_2$ @ 50Kg/ha. However, the same treatments were found to be comparable with soil application of $MgCl_2$ @ 25/75Kg per ha. The different levels of $MgCl_2$ were on par in their effect on seed yield. The highest BCR (4.68) was recorded with the soil application of $MgSO_4$ @ 25Kg/ha. There was a gradual increase in the oil content of castor seed for the soil application of Mg irrespective of the sources from 25 to 75 Kg ha^{-1}. The higher level of $MgCl_2$ and $MgSO_4$ each at 75 Kg/ha recorded higher oil content at 46.10 and 46.60% respectively. With Mg uptake, soil application of Mg irrespective of the sources and levels recorded higher values than the foliar application.

Keywords : Magnesium, Castor, Seed yield, Oil content.

INTRODUCTION

Castor (*Ricinus communis*. L.) occupies and important place in the vegetable oil economy of country. India accounts for 54% of global castor area and 51% of the words castor production and ranks first in the area and production in the world. India meets more than 70% of world requirement of castor oil its derivatives and earns more than Rs. 1000 crores annually through exports. It ideally suits dry land farming in *kharif* and with limited irrigations in post-monsoon both in traditional and non-traditional (Hegde, 2006).

In Tamil Nadu, farmers are growing hybrid castor as a pure crop both under rainfed and irrigated conditions and the farmers seldom apply imbalanced NPK fertilizers resulting in poor yields. In this context, application of magnesium in castor production definitely improves the productivity and quality. Magnesium plays a vital role in the plant as a plant nutrient. It occupies the centre-spot in the chlorophyll molecule and thus is indispensable for photosynthesis by plants. It is an activator of many enzymes involved in the carbohydrate metabolisms, synthesis of nucleic acids, phosphate transfer decarboxylations etc. Being a constituent of polyribosome, it takes part in protein synthesis (Biswas *et al.*, 1985).

The quantity of Mg needed differ for various plant species. Legumes were found to contain the highest percentages of Mg and small grains the lowest. Tufail Ahmed Khan *et al.*, (1990) reported that the per cent increase in yield over control varied from 86.5 to 22.6 per cent with soil application of 10 and 13 Kg ha^{-1} of Mg and S as $MgSO_4$ to sunflower, respectively. Foliar application of one per cent $MgSO_4$ along with 40 Kg N and 60 Kg P_2O_5 ha^{-1} at the time of sowing increased the seed yield and oil content of sunflower (Kene *et al.*, 1990). As there is almost less work on magnesium fertilization to castor especially Hybrid TMVCH I under irrigated condition, the present study has been undertaken to study the influence of different sources and levels of Mg on the yield and quality of castor.

MATERIAL AND METHODS

The field experiment with castor hybrid TMVCHI has been carried out to study the influence of different sources and levels of Mg on the yield and quality of castor adopting eleven treatments includes NPK - Recommended Dose of NPK fertilizers (60 : 30 : 30 Kg NPK/ha) alone and with $MgCl_2/MgSO_4$ @ 25, 50 & 75 Kg ha^{-1} as soil application and as 0.5% / 1.0% spray on 30, 60 & 90 DAS which were replicated thrice in RBD. The soil of the experimental site was sandy loam in texture with a pH of 6.62 and EC of 0.31 dSm^{-1}. The soil was low in

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available $KMnO_4$–N (216Kg ha^{-1}), low in Olsen–P (8.2 Kg ha^{-1}) and high in NH_4OAc–K (478 Kg ha^{-1}). All the treatments received an uniform dose of 60 : 30 : 30 Kg NPK/ha in the form of urea, super phosphate and muriate of potash respectively where N is applied in three splits *viz.*, 30 Kg N as basal and 15 Kg N each at 30 & 60 days after planting. The trials were terminated by recording the stalk weight of individual plots. The oil content is analysed as per George W. Priest & Von Mikusch (1940). Post harvest soil samples were collected and analyzed for available nutrients and the pooled three years data of 2006-08 were subjected to statistical analysis (Panse and Sukhatme, 1978). The benefit cost ratio for different treatment was worked out considering gross returns and cost of cultivation.

RESULTS AND DISCUSSION

The castor seed yield data (Table 1) showed a positive response for the application of magnesium irrespective of the sources. The seed yield of castor ranged from 3345 to 4165 Kg ha^{-1}, with respect to mode of application, the soil application of magnesium along with recommended NPK recorded significantly higher seed yield compared to foliar application @ 0.5/1.0 per cent concentration. Soil applicaton of $MgSO_4$ @ 25 Kg ha^{-1} recorded higher seed yield (4165 Kg ha^{-1}) followed by $MgCl_2$ @ 50 Kg ha^{-1}. However, the same two treatment were found to be comparable with soil application of $MgCl_2$ 25/ 75 Kg per ha and 50 Kg $MgSO_4$/ha. The different levels of $MgCl_2$ were on par in their effect of seed yield. The response for higher level of $MgSO_4$ *viz.*, 75 Kg/ha was found to be non-significant.The application of recommended level of NPK recorded the lowest yield of 3345 Kg ha^{-1} found to be comparable with foliar applied $MgCl_2$/$MgSO_4$ at 0.5 and 1.0% concentration. The highest BCR (4.68) was recorded in soil applicaction of $MgSO_4$ @ 25 Kg/ha followed by $MgSO_4$ @ 50 Kg/ha and 50 Kg $MgCl_2$/ha. The lowest BCR was recorded by the NPK applied plot (Table 1). Brain Whipker *et al.* (1998) suggested that the optional fertilizer ratio of N:P:K: Ca: Mg would be 8:1:10:4:2 for pot sun flowers. Adubacao *et al.* (2005) reported that chemical fertilization is an effective technique for increasing castor yield and fertilization with NPK and lime as source of calcium and magnesium increased the yield from 1.072 to 2.298 t ha^{-1} due to fertilization, in Northeast Brazil.

There was a gradual increase in the oil content of castor seed for the soil application of Mg irrespective of the sources from 25 to 75 Kg/ha. The effect of lower foliar applied magnesium was comparable irrespective of concentration and sources. The higher level of $MgCl_2$ and $MgSO_4$ each at 75 Kg/ha recorded higher oil content at 46.10 and 46.60% respectively (Table 1). Sudhir and Ananthanarayanan (1986) noted that there was a slight rise in the shelling and soil percentage of groundnut due to Mg application. However, the application of magnesium as soil application recorded higher magnesium availability in soil followed by foliar applied magnesium. Whereas NPK alone recorded lower Mg content in soil.

The soil application of $MgCl_2$ @ 50 Kg/ha recorded higher Mg uptake of 36.46 Kg/ha followed by $MgCl_2$ at higher level and all levels of $MgSO_4$ as soil application. However the lower level of $MgCl_2$ @ 25 Kg/ha was found to be on par with 1.0% foliar applied $MgCl_2$. The foliar concentrations (0.5/1.0 %) recorded on par values with NPK alone. Sudhir and Ananthanarayanan (1986) stated that magnesium application increased the uptake of NPK due to synergistic effect and also Mg regulates uptake of P as a carrier within the plant. Magnesium sulfate offered to the cotyledons caused a threefold increase of magnesium ions in the sieve-tube sap, which was not inhibited by K^+. It is concluded that phosphate availability is a decisive factor for mobilization and translocation of magnesium ions within the plant (Wenjun Zhong *et al.*, 1993).

CONCLUSION

It can be concluded that Castor responded well to the application of magnesium in combination with NPK. There was

Table 1 : Effect of magnesium fertilization on seed yield, oil content and soil properties after *Ricinus communis* L..

Treatments	Seed yield (Kg ha^{-1})	BCR	Oil content (%)	Avail. Mg (C mol Kg^{-1})	Total Mg uptake (Kg ha^{-1})
T_1 - NPK - Recommended Dose of NPK fertilizers (60:30:30 Kg NPK/ha)	3345	3.79	44.25	4.6	20.48
T_2 - NPK + $MgCl_2$ @ 25 Kg/ha as soil application	3927	4.27	45.15	6.2	31.08
T_3 - NPK + $MgCl_2$ @ 50 Kg/ha as soil application	4160	4.35	45.74	6.4	36.46
T_4 - NPK + $MgCl_2$ @ 75 Kg/ha as soil application	3930	3.96	46.10	6.4	36.35
T_5 - NPK + $MgSO_4$ @ 25 Kg/ha as soil application	4165	4.68	44.78	7.4	33.33
T_6 - NPK + $MgSO_4$ @ 50 Kg/ha as soil application	3980	4.44	45.34	7.2	34.41
T_7 - NPK + $MgSO_4$ @ 75 Kg/ha as soil application	3820	4.22	46.60	7.4	33.16
T_8 - NPK + 0.5% $MgCl_2$ on 30, 60 & 90 DAS	3495	3.94	45.17	5.5	26.67
T_9 - NPK + 1.0% $MgCl_2$ on 30, 60 & 90 DAS	3435	3.86	44.33	5.4	25.70
T_{10} - NPK + 0.5% $MgSO_4$ on 30, 60 & 90 DAS	3420	3.87	45.86	5.2	23.53
T_{11} - NPK + 1.0% $MgSO_4$ on 30, 60 & 90 DAS	3440	3.89	44.29	5.2	25.16
CD (p=0.05)	315	-	1.65	0.32	5.31

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a gradual increase in the oil content of castor seed for the soil application of Mg irrespective of the sources from 25 to 75 Kg/ha To achieve higher yield and benefit cost ratio in hybrid castor TMVCHI, basal application of $MgSO_4$@25 Kg ha^{-1} along with 60 Kg N, 30 Kg, P_2O_5 and 30 Kg K_2O ha^{-1} is highly essential under irrigated conditions.

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Influence of Some Heavy Metals on Seedling Physiology in *Lycopersicon esculentum* Mill

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Interaction of different concentrations of heavy metals such as Hg, Cd & Cr on seed germination, seedling growth , number of lateral roots, fresh and dry weight were recorded. Apart from effect on early seedling growth the present investigation was also extended to study some enzyme activities in germinating seeds of *Lycopersicon esculentum*. The effect of heavy metals on various attributes studied was vary with level of concentrations and durations of heavy metals. The data was represented in tables and figures and the whole data was subjected to statistical analysis.

INTRODUCTION

Tomato, *Lycopersicon esculentum* Mill., is highly valuable and nutritious food crop. It is one of the major vegetable crops grown in both tropical, sub-tropical and temperate regions.

Heavy metal pollution is one of the current most trouble some environmental problems due to wide spread use of metals for industrial and agricultural purpose (Nriagu and Pacuna, 1988; Fernandes and Henriues, 1991).

The presence of heavy metals in waste materials within the territory of mines and chemical industries causes chemical pollution of the soil and phytotoxic phenomena may develop causing severe alterations in plant structures and growth systems (Corradi *et al;* 1993). The toxic heavy metals entering the ecosystem may lead to geoaccumulation; bioaccumulation and biomagnification.

Mercury is an important non-degradable toxic heavy metal pollution and increasing rapidly in our biosphere and is accumulated by plants in polluted areas. Human activities are estimated to account for 2×10^4 to 7×10^4 tons of mercury per year being released into the atmosphere and water supply (Robinson and Tuovinen, 1984). Some workers observed toxic effects of mercury on germinating rice seeds (Mukherji and Ganguly, 1974) and on physiological aspects of the several legumes (Sharma, 1982).

Cadmium is one of the most hazardous elements for humanbeings. Cadmium is a major environmental contaminent of air, water and soil (Lagerwerff, 1967).

Chromium too is an important environmental contaminant released into the atmosphere due to its huge industrial use (Nriagu and Niboer, 1988). Chromium is one of the important essential element for plants and animals (Mertz and Schwartz, 1995). However, at higher concentration it becomes toxic to plants (Maywald and Weigel,1997), and animals and becomes carcinogenic in case of intense human exposure (Levis and Bianchi, 1982). Both the Cd and Cr inhibited the seed germination and seedling growth in *Solanum melangena* was reported by Neelima and Reddy (2002).

The present study was under taken to see the effect of some heavy metals on seedling physiology in Tomato plants.

MATERIAL AND METHODS

Healthy and uniform Tomato seeds were presoaked in distilled water for 2h and were surface sterilized with $HgCl_2$ for two minutes and washed thoroughly with distilled water. The seeds were then soaked in different concentrations of Mercuric chloride (5, 15, 35 and 50 ppm), Cadmium acetate (1000, 5000, 7000 and 10,000 ppm), Chromium (1000, 5000, 7000 and 10,000 ppm), individually for different durations of 6, 12 and 24h respectively. After the heavy metals treatment, seeds of each petriplate were rinsed with distilled water for 3 times and allowed to germinate in a petriplate lined with distilled water moistened filter paper. Seeds soaked in distilled water were constituted as control for all durations. The radicle emergence was considered as germination of seed for calculation of seed germination. The seedling growth was studied after laps of 15 days of experiment in the petriplate. The fresh and dry weights of the total seedling were also noted. For dry weight analysis seedlings were kept in oven for 48h at 60°C .The data was the average of 3 replicates and have been stastically analysed.

Biochemical changes in germinating seeds were analyzed in different concentrations of heavy metals at 48h of seed germination in terms of protein content (Lowry *et al.*, 1951), protease (Yomo and Verner, 1973) using casein as

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substrate and Amylase activity (Malik and Singh, 1980). Simultaneously controls were also maintained along with the treated samples at room temperature All experiments were done in triplicate and repeated twise and considered average data which represented in figures.

RESULTS AND DISCUSSION

The influence of different concentrations of Hg, Cd and Cr individually on germination of seed and seedling growth were represented in (Table 1).

The percentage of germination, seedling growth in all treatments showed concentration dependent inhibitory effect. High the concentration greater the inhibitory effect. However, exceptionally in higher concentration (10,000 ppm) for 24h duration of Cd caused to increase the root length and at Cr 7000 and 10,000pm treatment for 12h showed increased shoot length over control.

Lateral roots were not seen in control samples as well as all treated samples. However, induction of lateral roots were noticed in meager in their number in some of the Cr treatments. 7000 and 10,000ppm for 12h duration of Cr treatment showed induction of lateral roots. The number of lateral roots at 7000ppm was 0.23 and at 10,000ppm 0.19 (Table 2,3 & 4). Fresh and dry weights of total seedlings for all treatments showed positive correlation with the growth of the seedlings except in 7000 and 10,000ppm of Cr 12h (Fig.1-6).

The changes in biochemical activities were analyzed after 48h of seed germination in laboratory conditions and the data was represented in (Fig.7-15).

Protein content in all treatments were negatively affected with increasing concentration in all durations, exceptionally in treatment of Hg 5,15 & 35 at 6h, Cd 1000 & 5000ppm at 24h and Cr 5000, 7000 & 10,000ppm at 24h showed increase in protein content.

Protease activity showed decreasing trend in all most all treatments as the concentration increases in all durations except Cr treatment upto where increase in 7000ppm activity was noticed.

Amylase activity was decreased when compared to controls in Hg treated seeds except 5ppm treatment for 6h where amylase activity increased. However, the effect was in significant within the concentration and durations.

The Cr treated seeds showed decrease in the amylase activity at shorter durations (6h) but, in the samples at longer durations (12 and 24h) upto 5000ppm, there was an increase in the activity. Still in remaining higher concentrations there was decrease in activity except 12h for 10000ppm when compared to controls.

In the present study heavy metals Hg, Cd & Cr showed inhibition in seedling growth. Growth inhibition results of present investigation find support from work of Rauser (1978). Panda and Patra (1997) have noticed the great phytotoxicity of Cr in plants causing reduction in growth, yield and enzyme activities. Heavy metal toxicity is primarily associated with alteration of root physiology, thereby inhibiting the root elongation (Woolhouse, 1983).

Table 1 : Effect of Hg, Cd and Cr on seed germination in Tomato.

Presoaking treatment in hours Mercury	Concentration in ppm	% of germination	Presoaking treatment in hours Cadmium	Concentration in ppm	% of germination	Duration in hours Chromium	Concentration in ppm	% of germination
6	Control	95.0	6	Control	95.0	6	Control	95.0
	5	83.0		1000	85.0		1000	90.0
	15	80.0		5000	83.0		5000	84.0
	35	71.0		7000	75.0		7000	79.0
	50	60.0		10000	69.0		10000	70.0
12	Control	95.0	12	Control	95.0	12	Control	95.0
	5	81.0		1000	86.0		1000	89.0
	15	79.0		5000	71.0		5000	80.0
	35	67.0		7000	62.0		7000	76.0
	50	60.0		10000	57.0		10000	69.0
24	Control	95.0	24	Control	95.0	24	Control	95.0
	5	63.0		1000	70.0		1000	73.0
	15	51.0		5000	61.0		5000	64.0
	35	42.0		7000	50.0		7000	57.0
	50	37.0		10000	41.0		10000	45.0

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Table 2 : Effect of Mercury on seedling growth, fresh and dry weight of 15 days old tomato seedlings.

Presoaking treatment in hours	Concentration in ppm	Root length (cm)	Shoot length (cm)	Total length (cm)	Initiation of lateral roots	Total weight of 10 seedlings (mg)	
						Fresh	Dry
6	Control	4.2 + 0.341	5.4 + 0.621	9.6 + 0.170	-	206 + 0.813	24 + 0.381
	5	3.81 + 0.318	5.01 + 0.405	8.8 + 0.527	-	198 + 702	23 + 0.452
	15	3.21 + 0.313	4.68 + 0.405	7.8 + 0.238	-	180 + 0.432	22 + 0.473
	35	3.16 + 0.303	4.2 + 0.585	7.3 + 0.435	-	178 + 1.032	18 + 0.368
	50	2.8 + 0.321	3.85 + 0.725	6.6 + 0.574	-	175 + 0.489	15 + 0.371
12	Control	4.2 + 0.341	5.4 + 0.621	9.6 + 0.170	-	206 + 0.813	24 + 0.381
	5	3.56 + 0.298	4.9 + 0.395	8.4 + 0.152	-	178 + 1.032	21 + 0.9812
	15	3.0 + 0.338	4.31 + 0.134	7.3 + 0.435	-	167 + 1.108	17 + 0.295
	35	2.7 + 0.535	3.9 + 0.513	6.6 + 0.574	-	161 + 0.735	15 + 0.1697
	50	2.48 + 0.584	3.66 + 0.708	6.1 + 0.326	-	150 + 0.320	13 + 0.042
24	Control	4.2 + 0.341	5.4 + 0.621	9.6 + 0.170	-	206 + 0.813	24 + 0.381
	5	2.61 + 0.498	3.8 + 0.627	6.4 + 0.728	-	150 + 0.324	18 + 0.364
	15	2.26 + 0.256	3.53 + 0.659	5.7 + 0.532	-	144 + 0.563	14 + 0.107
	35	1.91 + 0.226	2.81 + 0.603	4.7 + 0.525	-	130 + 0.643	10 + 0.216
	50	1.58 + 0.211	2.4 + 0.482	3.9 + 0.3000	-	122 + 0.678	8 + 0.137

Table 3 : Effect of Cadmium on seedling growth, fresh and dry weight of 15 days old tomato seedlings.

Presoaking treatment In hours	Concentration in ppm	Root length (cm)	Shoot length (cm)	Total length (cm)	Initiation of lateral roots	Total weight of 10 seedlings (mg)	
6	Control	4.2 + 0.341	5.4 + 0.621	9.6 + 0.170	-	206 + 0.813	24 + 0.381
	1000	3.9 + 0.300	5.2 + 0.553	9.1 + 0.230	-	200 + 0.542	22 + 0.473
	5000	3.61 + 0.421	4.81 + 0.313	8.4 + 0.152	-	198 + 0.702	20 + 0.019
	7000	3.3 + 0.877	4.6 + 0.276	7.9 + 0.238	-	190 + 0.470	18 + 0.368
	10000	3.0 + 0.697	4.2 + 0.341	7.2 + 0.331	-	187 + 0.213	16 + 0.075
12	Control	4.2 + 0.341	5.4 + 0.621	9.6 + 0.170	-	206 + 0.813	24 + 0.381
	1000	4.0 + 0.276	5.2 + 0.553	9.2 + 0.208	-	187 + 0.213	20 + 0.019
	5000	3.1 + 0.690	4.2 + 0.341	7.3 + 0.435	-	178 + 1.032	17 + 0.108
	7000	2.81 + 0.603	3.51 + 0.545	6.3 + 0.702	-	167 + 1.108	14 + 0.082
	10000	2.5 + 0.718	3.21 + 0.771	5.7 + 0.532	-	161 + 0.735	10 + 0.26
24	Control	4.2 + 0.341	5.4 + 0.621	9.6 + 0.170	-	206 + 0.813	24 + 0.381
	1000	3.21 + 0.771	4.9 + 0.395	8.1 + 0.129	-	183 + 0.531	21 + 0.602
	5000	2.6 + 0.765	3.51 + 0.545	6.1 + 0.326	-	144 + 0.563	16 + 0.075
	7000	2.41 + 0.494	3.1 + 0.619	5.5 + 0.585	-	140 + 0.614	13 + 0.120
	10000	4.9 + 0.3925	2.9 + 0.493	7.8 + 0.238	-	130 + 0.643	11 + 0.037

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Table 4 : Effect of Chromium on seedling, growth fresh and dry weight of 15 days old tomato seedlings.

Presoaking treatment in hours	Concentration in ppm	Root length (cm)	Shoot length (cm)	Total length (cm)	Initiation of lateral roots	Total weight of 10 seedlings (mg)	
6	Control	4.2 + 0.341	5.4 + 0.621	9.6 + 0.170	-	206 + 0.813	24 + 0.381
	1000	4.0 + 0.012	5.3 + 0.671	9.4 + 0.650	-	202 + 0.821	21 + 0.602
	5000	4.16 + 0.301	5.4 + 0.621	9.5 + 0.509	-	200 + 0.542	20 + 0.019
	7000	3.9 + 0.300	5.33 + 0.596	9.2 + 0.208	-	198 + 0.702	18 + 0.368
	10000	3.6 + 0.516	5.1 + 0.680	8.7 + 0.129	-	190 + 0.470	17 + 0.295
12	Control	4.2 + 0.341	5.4 + 0.621	9.6 + 0.170	-	206 + 0.813	24 + 0.381
	1000	4.9 + 0.395	5.2 + 0.553	10.1 + 0.504	-	190 + 0.470	22 + 0.473
	5000	4.2 + 0.341	5.1 + 0.680	9.3 + 0.173	-	183 + 0.531	18 + 0.364
	7000	4.06 + 0.268	5.5 + 0.585	9.5 + 0.509	0.23 + 0.0066	210 + 0.645	26 + 0.401
	10000	3.5 + 0.704	5.6 + 0.372	9.01 + 0.230	0.19 + 0.005	208 + 0.421	25 + 0.429
24	Control	4.2 + 0.341	5.4 + 0.621	9.6 + 0.170	-	206 + 0.813	24 + 0.381
	1000	3.8 + 0.294	5.1 + 0.680	8.9 + 0.500	-	187 + 0.213	21 + 0.602
	5000	3.41 + 0.614	4.83 + 0.363	8.2 + 1.122	-	161 + 0.735	17 + 0.108
	7000	2.8 + 0.521	4.5 + 0.230	7.3 + 0.435	-	144 + 0.563	14 + 0.082
	10000	2.35 + 0.368	4.16 + 0.314	6.5 + 0.616	-	122 + 0.678	12 + 0.0256

Fig. 1 : Effect of presoaking sowing treatment Mercury for 6, 12 and 24h on seedlings fresh weight in *Lycopersicon esculentum*

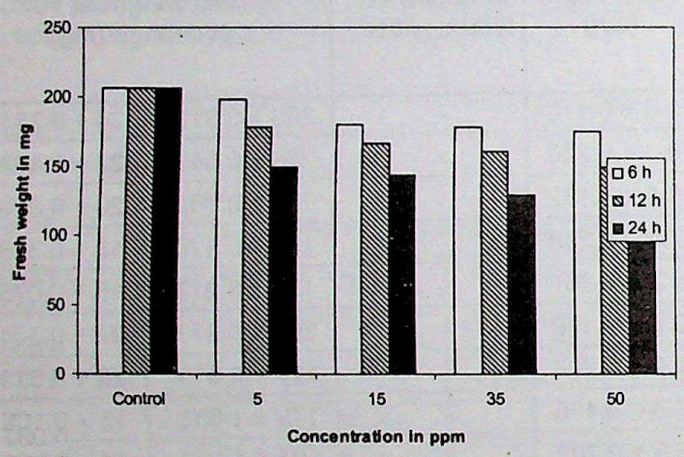

Fig. 2 : Effect of presoaking sowing treatment Cadmium for 6, 12 and 24h on seedlings fresh weight in *Lycopersicon esculentum*

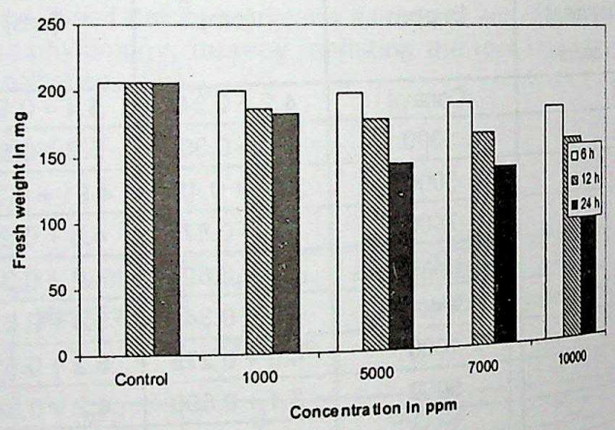

Fig. 3 : Effect of presoaking sowing treatment Chromium for 6, 12 and 24h on seedlings fresh weight in *Lycopersicon esculentum*

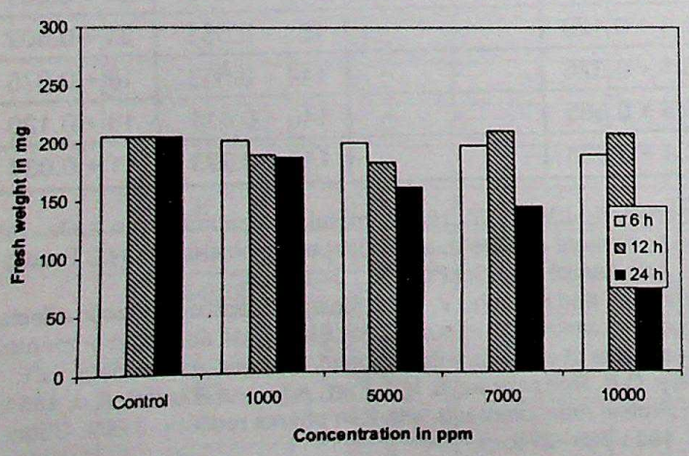

Fig. 4 : Effect of presoaking sowing treatment Mercury for 6, 12 and 24h on seedlings Dry weight in *Lycopersicon esculentum*

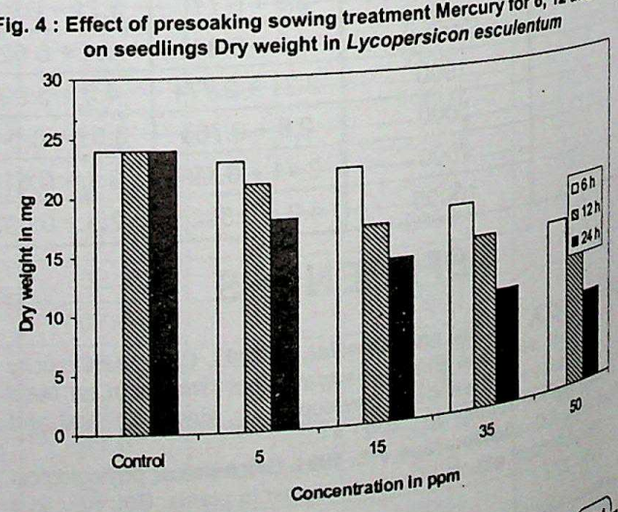

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Fig. 5 : Effect of presoaking sowing treatment Cadmium for 6, 12 and 24h on seedlings Dry weight in *Lycopersicon esculentum*

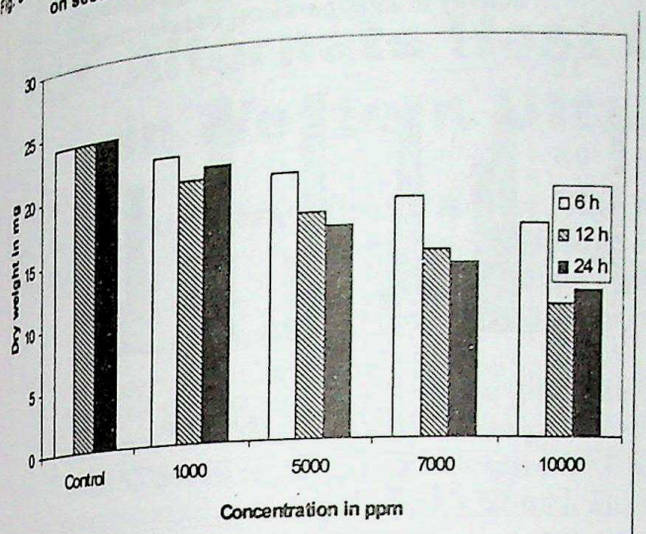

Fig. 6 : Effect of presoaking sowing treatment Chromium for 6, 12 and 24h on seedlings Dry weight in *Lycopersicon esculentum*

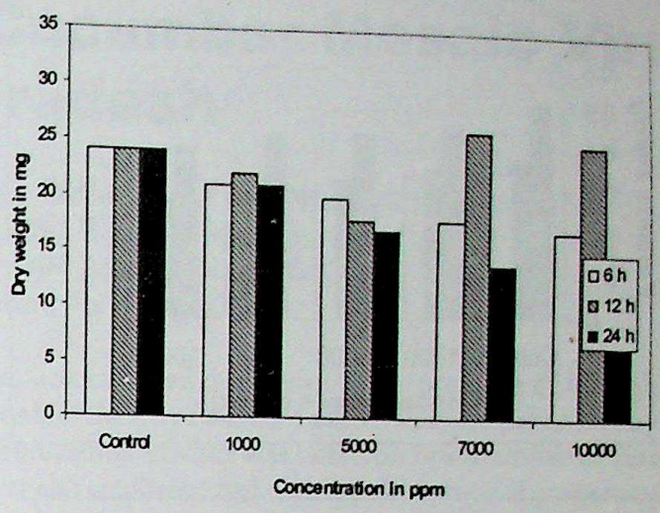

Fig. 7 : Effect of Mercury on protein content in *Lycopersicon esculentum*

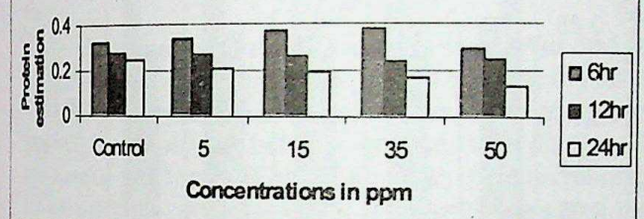

Fig. 8 : Effect of Cadmium on protein content in *Lycopersicon esculentum*

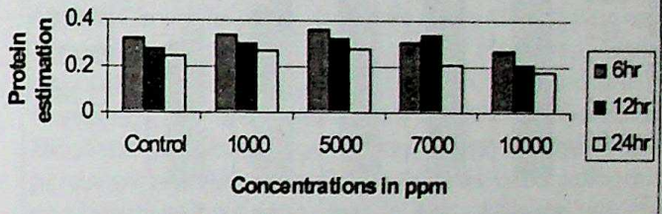

Fig.9:Effect of Chromium on protein content in *Lycopersicon esculentum.*

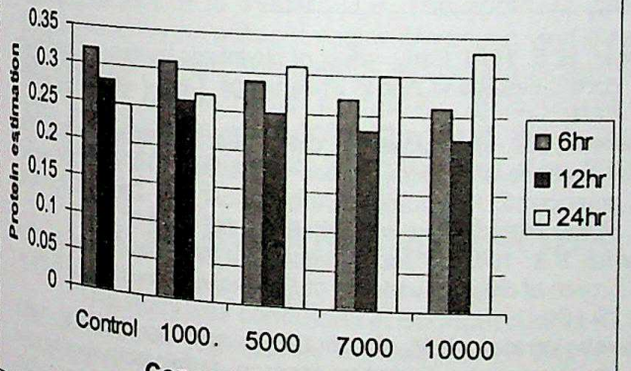

Fig.10:Effect of Mercury on protease activity in *Lycopersicon esculentum.*

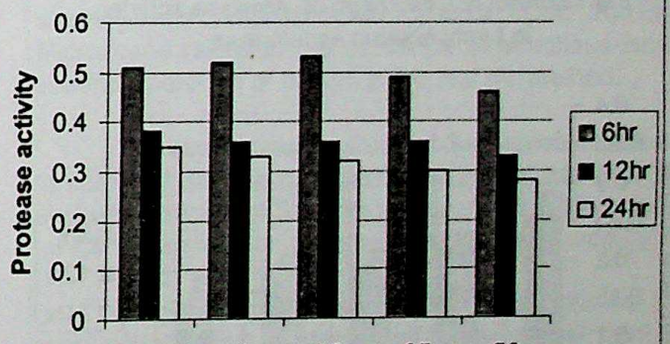

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Fig.11:Effect of Cadmium on protease activity in *Lycopersicon esculentum*.

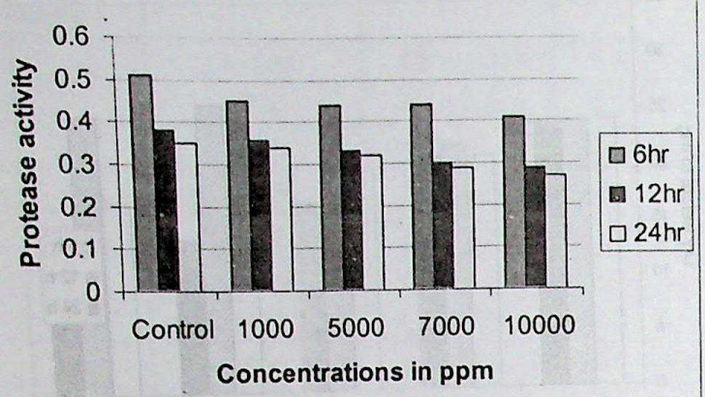


Fig.12:Effect of Chromium on protease activity in *Lycopersicon esculentum*.

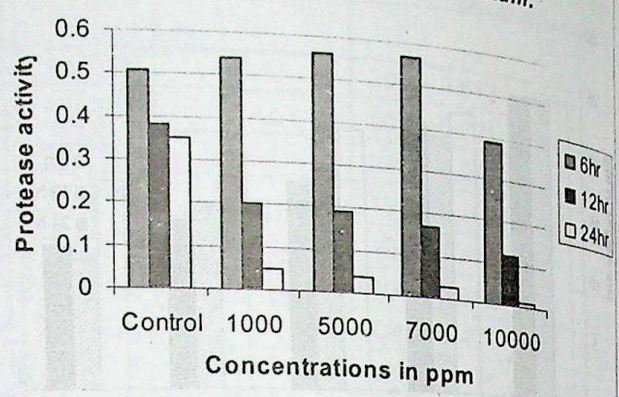


Fig.13:Effect of Mercury on Amylase activity in *Lycopersicon esculentum*.

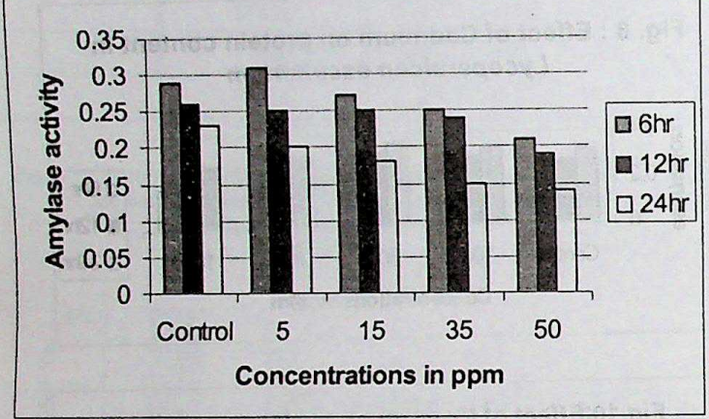


Fig.14:Effect of Cadmium on Amylase activity in *Lycopersicon esculentum*.

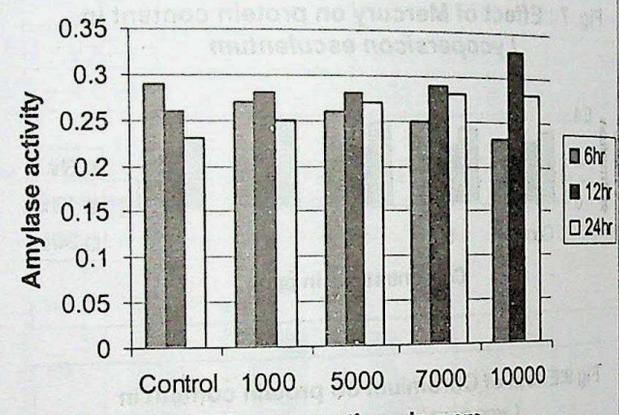
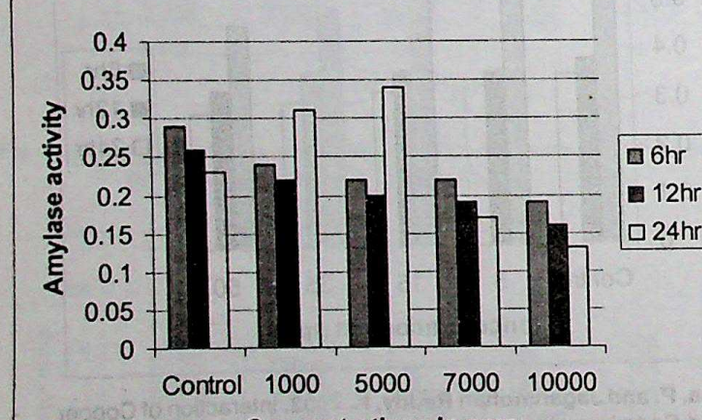


Fig.15:Effect of Chromium on Amylase activity in *Lycopersicon esculentum*.



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Solanaceous Weed (*Solanum nigrum*) as an Alternate Host of Cucumber Mosaic Virus in Western Uttar Pradesh

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A mosaic disease affecting *S. nigrum* (Black nightshade) was collected to determine the presence of virus in cucumber (*C. sativus*). The infected sample tested positive for CMV by Enzyme Linked Immunosorbent Assay (ELISA). Solanaceous weed was positive for CMV. Identification of virus was based on host reaction *in vitro* properties and particle morphology (28 nm). The virus was also confirmed by host range and physical properties.

Keywords : Weed, *Solanum nigrum* (black nightshade) CMV, Cucumber.

INTRODUCTION

Volunteer plant and weeds provide shelter and source of nutrients for virus vectors. Besides facilitating the spread of disease as alternative source of inoculum, these plants, sustain the viability of the virus between crop seasons (Duffus, 1964). Numerous plant species of the weeds that act as reservoir for a virus and its vector including the genus *Solanum nigrum,* have been found to be important in the epidemiology of certain viral diseases.

S. nigrum is commonly found among irrigated summer crops of cucumber in western U.P. The aphid. *M. persicae* (Sulzer) which is an efficient vector of various viral diseases colonizes in *S. nigrum*. The weed *S. nigrum* is susceptible to a wide range of viruses (Haider *et al.,* 2007, 2005; Gracia *et al.,* 2006; Chatzivassiliou *et al.,* 2007; Chen *et al.,* 2004; Coutts *et al.,* 2006; Boubourakes *et al.,* 2004; Kazinczi *et al.,* 2006).

MATERIAL AND METHODS

This study was conducted in Baghpat district (U.P.) at various fields of vegetables. Samples of *S. nigrum* were collected from different locations exhibiting virus-induced symptoms (mosaic, mottling and stunting) in 2006-2008. The presence of viruses in the samples was confirmed by DAS-ELISA, host range and *in vitro* properties. Insect transmission studies were carried out with aphid *M. persicae*.

RESULTS AND DISCUSSION

Naturally infected *S. nigrum* found in cucumber field showed mosaic mottling symptom, inoculating on cucumber (*C. sativus*) leaves show mild mosaic symptoms 8-10 following inoculation. The virus was transmitted by mechanical means to serveral host *viz., C. amaranticolor, C. murale, Benincasa hispid, Cucurbita pepo, Cucumis maxima, Citrulus vulgaris, Lagenaria ciceria, Luffa acutangula, L. cylindrica, Momordica charantia, Triocosanthes anguiana, Capsicum annum. C. frutescens, Datura stramanium, Nicandra physaloidis* and *Nicotiana glutinosa. M. persicae* transmitted the virus in non persistant manner when *C. sativus* was used as both cloner and recipient host. The virus in sap of *C. sativus* retained infectivity it was diluted to 10^{-3}, heated at 65°C for 10 min. and stored for 4 days at ambient temperature (28-30°C). *S. nigrum* in cucumber field samples were tested for the presence of virus by ELISA test, using antiserum PVY, TSWV, PRSV-P, PRSV-W, Geminivirus, AMV and CMV. Most plants of *S. nigrum* showing sings of viral attack were positive of CMV. The Electron Microscope revealed the presence of infectious isometric spherical particles of 28 mm in the leaf dip method.

The aim of present study of Solanaceous weeds was to confirm their role as possible source of the virus and to evaluate the ability of *Solanum nigrum* plant to severe as source for CMV transmission by aphids for cucumber crop.

The symptom exhibited on leaves of *Solanum nigrum* and Cucumber due to infection of this virus resembled that of CMV (Bidar and Reddy, 1990, George *et al.,* 1993, Singh, 1993, Kirmani *et al.,* 1997).

Physical properties of the virus under study resembled with CMV isolate reported earlier in various crop plants (Bidari and Reddy, 1990 and Kirmani *et al.,* 1997).

The host range of the virus study was restricted to Chenopodiaceae Cucurbitaceae, Leguminaceae, Solanaceae and Brassicae and resembled those reported by Hobbes *et al.,* (2000). The host range of this virus resembled as reported

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Fig. 1 : *Solanum nigrum* plants showing mosaic mottling, downward curling symptoms due to CMV infection.

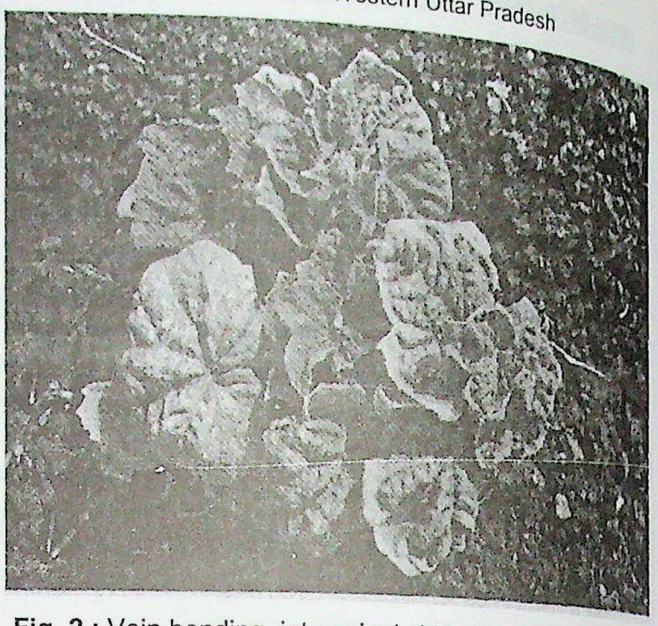

Fig. 2 : Vein bending, interveinal chlorosis, mosaic and leaf distortion in *Cucumis sativa* due to CMV infection.

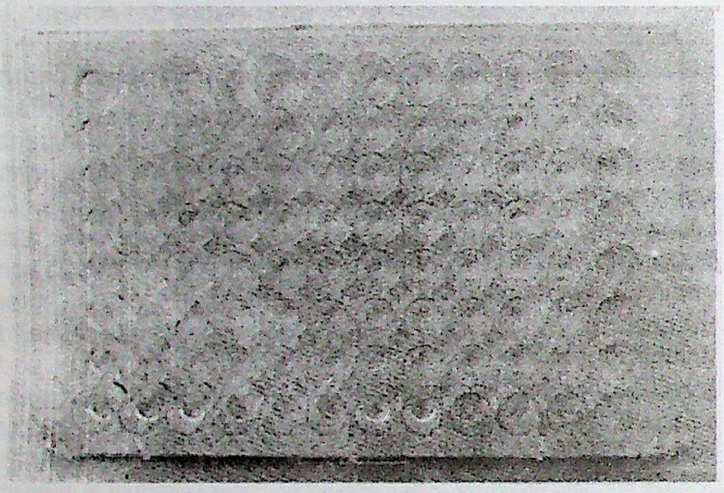

Fig. 3 : ELISA plate showing positive reaction (yellow colour) of infected weed *S. nigrum* with CMV antiserum.

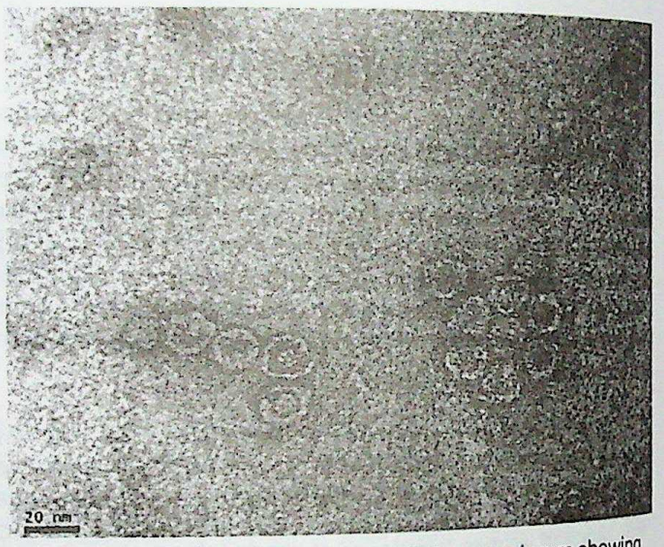

Fig. 4 : Electron microscopy of *Solanum nigrum* showing Isometric particles of CMV

by (Wellman, 1934, Simons, 1957a; Anjaneyulu and Appa Rao, 1967; Prasad Rao, 1976; Sharma *et al.,* 1984; Fujisawa *et al.,* 1986; Rishi and Dhawan, 1989; Bidar and Reddy, 1990; Green and Kim, 1991; George *et al.,* 1993; Singh, 1993; Kirmani *et al.,* 1997 and Srivastava *et al.,* 1992) except for its being able to infect members of family Brassicaeae. However, the present virus differs from CMV reported by Wellman (1934), Simons (1957) and Anjaneyulu and Appa Rao (1967) in its ability to infect *Zinnia elegans*.

The virus under study induced systemtic symptoms on *Cucumis sativus*. In this respect it resembled that of Cucumber mosaic Cucumovirus reported from India on different crops. (Green and Kim, 1991; George *et al.*, 1993; Singh, 1993 and West Malaysia (Fujisawa *et al.*, 1986 and Green and Kim, 1991). *Cucumis sativus* has been reported as a diagnostic / differential host of CMV (Prasad Rao, 1976, Bidar and Reddy, 1990, Green and Kim, 1991). The present virus differs in host range from all other viruses (except CMV) reported on cucumber.

In ELISA, the virus under study reacted positively with antiserum against CMV and negatively with other antisera. These results are similar to reported by Soledad Sacristan *et al.*, 2004 and Hobbes *et al.*, 2000.

The leaf dip preparation of present virus revealed the presence of isometric particles measuring on an average of 28 nm in diameter. In this respect it resembled with Cucumber mosaic virus Cucumovirus (Sharma *et al.*, 1984; Green and Kim, 1991).

On the basis of biological properties, serology and particle morphology, the virus was identified as cucumber mosaic virus (CMV). *Solanum nigrum*, a common weed plant might act as a reservoir of CMV by providing inoculum for

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economically important crop like cucumber, which is susceptible to CMV. This is first time reported in India.

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Performance of Rice and Pulse Varieties at Different Soil ESP Levels

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Field experiments were conducted to study the performance of rice varieties and pulse crop at varying artificially created sodicity level at Anbhil Dharmalingam Agricultural College and Research Institute, Trichy, Tamil Nadu. At higher Exchangeble Sodium Percentage (ESP) level of 35 in rice varieties, the magnitude of decrease in grain yield was less in TRY 1 and TRY 2 while the percentage of yield reduction was highest in pulses (70%). Based on yield reduction at higher ESP levels it can be concluded that the rice varieties *viz.*, TRY-1 and 2 are tolerant varieties for cultivation in high ESP sodic soil.

Keywords : Exchangeable Sodium Percentage (ESP), Rice varieties, Yield, BC ratio.

INTRODUCTION

Sodicity is one of the limiting factors for achieving optimum crop yields in vast area of our country. Sodic soils (ESP > 15) are of paramount importance due to their large extent and provide a scope for the horizontal expansion of the productivity through increased area. Sodic soil suffer from low productivity primarily by imbalanced nutrient availability and secondly due to unfavourable physical condition of the soil. Though technologies are available for reclamation of sodic soils, growing tolerant crop is also one of the recommended technologies. In Tiruchirappalli district about 20,000 ha are salt affected soils. These salt affected soils are used to raise single rice crop using canal water followed by pulses or some other short duration crops. Keeping this fact in view the tolerance of rice varieties and pulse crop was evaluated under the scheme on ICAR AICRP on management of salt affected soils.

MATERIAL AND METHODS

The field experiments were conducted in Anbil Dharmalingam Agricultural College and Research Institute. Tiruchirappalli, Tamil Nadu Agricultural University during the year 2002-04. This study was laid out in split plot design with different ESP levels (8,16, 26 and 35) as main plot treatment and different varieties/ crops as subplot treatments and replicated thrice. Different ESP levels of 26 and 35 were created from the inherent ESP of 16 by adding 15 and 29 Kg of $NaHCO_3$ per plot respectively while ESP of 8 was created by adding required quantity of gypsum (12 Kg/plot). The soil is clayloam in texture and taxonomically belonged to fine, mixed calcareous isohyperthermic Vertic Ustropept with pH of 8, 6, EC 0.43 dS/m and ESP of 16. Rice varieties - TRY-1, Co - 43 and TRY (R) - 2 during *rabi* season, black gram (T9 and ADT - 5) and green gram (Pusabold) during *kharif* season, and again rice varieties *viz.*, ADT - 39, ADT - 45 and White ponni during next *rabi* season were evaluated. Nitrogen, Phosphorus and Potassium were applied as per the recommendation. The crops were harvested at maturity and plotwise yield was recorded.

RESULTS AND DISCUSSION

Yield

Rice : The yield of different rice varieties is given in Table 1. During *rabi* season the rice variety TRY 1 recorded the highest grain yield of 6767 Kg ha^{-1} at 16 soil ESP level which was significantly higher than that of 8 soil ESP level. There after yield decline was recorded. In TRY (R) - 2, the yield was no par up to 26 ESP and higher yield was recorded at 26 ESP. In Co - 43, the highest grain yield of 6492 Kg ha^{-1} was recorded at 16 ESP, which was on par with 8 ESP and declined thereafter. Garg and Srivastava (1986) observed pronounced yield reduction of sugarbeet at 32 ESP level. Sharma and Yadav (1988) reported decreased rice grain yield to the tune of 13% at at 45 ESP.

Pulses : The result on seed yield of pulse crop as influenced by different ESP levels is presented in Table 2. The data indicates that sodicity levels significantly influenced the seed yield. The highest yield of 963 and 1260 Kg ha^{-1} was obtained in black gram variety T9 and green gram variety Pusablod respectively at 16 ESP which was on par with 8 ESP. In black gram ADT 5, the highest yield (1333 Kg ha^{-1}) was recorded at 8 ESP. In both black gram and green gram yield decline was observed beyond 16 ESP. Such reduction in yield of chickpea at different ESP levels was also reported by Mishra (2001). The higher Sodium uptake might have interfered with plant nutrient and water uptake and plant metabolism which inturm resulted in lower yield at higher ESP levels. (Anand Swarup

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Table 1 : Effect of different ESP levels on grain yield of rice varieties.

ESP levels	Grain Yield (Kg ha^{-1})			
	TRY - 1	Co - 43	TRY (R) - 2	Mean
8	5775	6458	3633	5289
16	6767	6492	3642	5633
26	6042	5033	4108	5061
35	5178	4166	3633	4326
Mean	5941	5537	3754	
CD (5%)	M S	S at M	M at S	
	316 252	509	514	

Table 2 : Effect of different Soil ESP levels on pulse grain yield (Kg ha^{-1})

ESP levels	Seed Yield (Kg ha^{-1})			
	Black gram		Green gram	
	Var. T9	Var. ADT 5	Var. Pusa bold	Mean
8	907	1333	1243	1161
16	963	1177	1260	1133
26	333	457	523	438
35	293	393	440	376
Mean	624	840	867	
CD (P = 0.05)	M	S	M at S	S at M
	57.7	9.4	67.3	74.7

Table 3 : Effect of different Soil ESP levels on grain yield of rice.

ESP levels	Grain Yield (Kg ha^{-1})			
	ADT-39	ADT-45	White Ponni	Mean
8	3100	2600	3666	3122
16	3383	2766	3500	3216
26	2491	2508	3466	2822
35	2683	2525	3066	2758
Mean	2914	2600	3425	
CD (P = 0.05)	M	S	M at S	S at M
	239.0	135.0	324.8	270.9

2004). This result is in good accordance with the findings of Singh and Abrol (1986) who reported decreased yield and yield attributing parameters in soybean.

Rice : During second year three rice varieties *viz.*, ADT 39, 45 and Whiteponni were evaluated. The yield at 8 and 16 ESP were onpar and recorded the highest rice grain yield while the higher ESP level of 26 and 35 were onpar and recorded the lowest yield (Table 3). Among the varieties white ponni registered the highest yield followed by ADT 39 and 45. The variety ADT 39 showed drastic reduction in yield at 26 and 35 ESP while shiteponni registered considerable yield reduction beyond 26 ESP. The reduction in yield of tomato, cabbage, garlic, onion and Indian mustard due to alkalinity hazard was also reported (Anon 2002). This corroborates with findings of Singh *et al.*, (1992) who reported reductiion in height, girth and drymatter yield in different tree species. High concentration of Na than Ca on exchange complex results in adverse physical properties and reduction in the availability of several nutrients which caused nutritional disorder and lower yield (Anand Swarup 2004).

There was significant yield reduction in rice grain yield with raise in soil ESP and the per cent yield reduction at higher sodicity varied from 10 to 36. In pulses it varied from 58 to 65 and 65 to 71 respectively at 26 and 35 ESP. A rise in Na and the simultaneous fall in K and N contents due to increase in ESP is the main reason for greater reduction in grain yield. The lower crop yield in such soil are mainly due to sodium toxicity.

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Table 4 : B : C ratios for different crops at different ESP levels.

Crop / variety	ESP levels			
	8	16	26	35
Rice				
TRY 1	1.90	2.25	2.0	1.75
Co-43	2.15	2.15	1.70	1.40
TRY 2	1.20	1.25	1.40	1.20
ADT-39	1.20	1.25	0.95	1.05
ADT-45	1.10	1.00	0.90	0.80
White ponni	2.05	1.95	1.90	1.70
Pluses				
Blackgram T9	1.65	1.75	0.60	0.50
Blackgram ADT 5	2.40	2.15	0.85	0.70
Greengram Pusa bold	2.25	2.30	0.95	0.80

BC ratio : The BC ratio arrived for different crops at different ESP levels is given in Table 4. The rice varieties TRY-1, white ponni and Co 43 recorded higher BC ratios followed by other rice varieties and pulses. Pulse crop are not remunerative at higher ESP levels. Among the rice varieties, at higher soil ESP levels the percent yield reduction was low in TRY-1 adn the BC ratio was also found to be high.

It can be concluded from the study that TRY 1 and 2, Co 43 showed highest tolerance to exchangeable sodium (up to the level of 26). Next alternate is whiteponni which is having higher marker preference due to its fine grain nature. Since pulses are sensitive to sodicity it can be grwon when the ESP of soil is brought down to about < 16.

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Effect of Drip Fertigation on Growth and Yield of Chilli (*Capsicum annuum* L.)

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Field experiments were carried out at the Orchard of Agricultural College and Research Institute. Madurai, Tamil Nadu during *Kharif* 2007 and summer 2008 to assess the growth and yield potential of chilli under drip fertigation. The study revealed that, drip fertigation with 100 per cent water soluble fertilizer along with bio stimulants recorded highest plant height, number of primary and secondary branches, number of flower/plant and yield attributing characters and pod yield in chilli, cv. KKM-1.

Keywords : Drip fertigation, WSF, Bio stimulants, growth, yield Chilli.

INTRODUCTION

Chilli (*Capsicum annuum* L.) is a spice cum vegetable crop belonging to the family Solanaceae. Chillies are native of Peru and Mexico and the Portuguese towards the end of the 15th century first introduced it into India. Its cultivation became popular in the 17th century. India stands first in chilli cultivation in the world, covering 45 per cent of the total area. It is grown throughout the country in almost all the states, over an area of 4,4 lakh hectares with a production of 9.19 lakh tonnes of green chillies. In Tamil Nadu, it is grown in area of 0.68 lakh hectares with in annual production of 0.40 lakh tonnes. India earns nearly Rs. 210 crores of foreign exchange annually by exporting chillies.

Application of water soluble fertilizers through drip fertigation system is gaining Importance in present day agriculture to boost the production and productivity of various crops. However, the cost of water soluble fertilizer is very high which restricts the use of fertigation, it is essential to explore the possibility of using normal fertilizer in drip fertigation (Jeyabal *et al.*, 2000). Further , chilli crop requires a balanced fertilizer management for normal growth and development of the crop. It is a heavy feeder and high yield can be sustained only through the application of nutrients at optimum doses in balanced proportion. The nutrient requirement also varies with the growth stages of the crop. Among the various factors responsible for higher yield, the use of appropriate quantity of fertilizer at proper time plays a vital role in enhancing the productivity. Hence, planning the nutrient supply to the physiological stages of growth development, and depending on the soil and climatic characteristics result in high yields with quality produce. Proper scheduling must be planned to provide nutrients at a time when it required by plants (Suojala-Ahlfors *et al.*, 2005). Under drip irrigation, the spatial distribution of soil moisture and consequently crop roots is restricted to a small volume of soil directly below the emitters. Such a restriction has important implications for optimum fertilizer placement (Selvakumar, 2006). Taking these aspects into consideration, the present investigations was undertaken.

MATERIAL AND METHODS

The genetically, pure seed of chilli cv. KKM-I obtained from Agricultural College and Research Institute, Killikulam formed the base material for the study. The experiment was laid out in Randomized block design with nine treatments replicated thrice with plot size of 45 m^2 (10 m x 4.5 m). After uniform leveling, beds were prepared at a distance of 1.5 m and plants were transplanted uniformly with a spacing of 60/90 cm between two pairs, 45 cm between plants were followed.

Treatment details

T_1 = RDF through soil application (120 : 60 : 30 Kg NPK ha^{-1})
T_2 = 75% RDF as Urea + DAP (as basal) + KCl
T_3 = 75% RDF as Water Soluble Fertilizers.
T_4 = 100% RDF as Urea + DAP (as basal) + KCl
T_5 = 100% RDF Water Soluble Fertilizers.
T_6 = T_2 + liquid biofertilizers + Panchakavya + Humic acid
T_7 = T_3 + liquid biofertilizers + Panchakavya + Humic acid
T_8 = T_4 + liquid biofertilizers + Panchakavya + Humic acid
T_9 = T_5 + liquid biofertilizers + Panchakavya + Humic acid

Note :

- Water soluble fertilizers : Poly feed (19 % N, 19 % P and 19 % K) MAP (12 % N and 61 % P) and KNO_3 (13 % N and 45 % K).
- Liquid biofertilizers (Azophosmet 200 ml/acre), Panchakavya (10 litre/acre) and Humic acid (2 litre/acre) (TNAU recommendations).

Fertigation was scheduled once in three days starting from second week after planting. Each plot consists of three laterals for irrigating six rows of crops. A tap is provided at beginning of each lateral for giving controlled fertigation. The required quantity of N, P and K fertilizers as urea. MAP and

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KNO_3 as per the treatment were dissolved separately in 5 litres of water can to the each laterals of the individual plot. The data obtained from the present investigations were subjected to statistical analysis (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The highest plant height (78.01 cm and 77.10 cm at 180 DAT), number of primary branches (8.04 plant^{-1} and 7.92 plant^{-1} at 180 DAT), number of scondary branches (21.85 plant^{-1} and 19.62 plant^{-1} at 180 DAT). fruit length (11.31 cm and 11.02 cm), fruit girth (3.36 cm and 3.29 cm), number of flowers / plant (319.56 plant^{-1} and 338.14 plant^{-1}), number of fruits / plant (260.11 plant^{-1} and 243.29 plant^{-1}), fresh fruit yield/ plant (510.24 g plant^{-1} and 462.47 g plant^{-1}) dry pod yield (182.25 g plant^{-1} and 174.25 g plant^{-1}) and dry pod yield (4.05 t/ha and 3.91 t/ha) recorded in application of 100 per cent water soluble fertilizer along with bio stimulants (T_9) significantly influenced the growth and yield parameters of chilli at all crop growth stages both in *Kharif* and summer season.

The results revealed that the plant height registered during different stages of crop growth significantly differed with the treatments like (T_9) recorded the highest plant height of 78.01 cm and 77.10 cm at 180 DAT in both *kharif* and *summer* season respectively (Table 1). The increased plant height under T_9 was mainly due to the continuous availability of required quantity of water along with the required nutrients. These results are in agreement with the findings of Muralidhar (1998) and Aruna *et al.,* (2007) who also reported that the increased plant height was due to better utilization of applied nutrients and water by the plants under drip fertigation.

More number of primary branches were recorded in the treatment of (T_9) (8.04 plant^{-1} in *kharif* and 7.92 plant^{-1} in *summer* at 180 DAT). Lower number of primary branches were recorded in plant received recommended dose of fertilizers as soil application (T_1) in both *kharif* and *summer* season. Similarly (T_9) showed more number of secondary branches of (21.85 plant^{-1} in *kharif* and 7.92 plant^{-1} in *summer* at 180 DAT) (Table 2 & 3). The increase in number of secondary branches per plant might be due to higher availability of N, P and K during early stages of crop growth, which would have increased the root activity, where the synthesis and sustainability of nutrients during cytokining take place. The transport of cytokinin from the root would have encouraged auxiliary buds resulting in increased number of branches (Pandey *et al.,* 1996). Similar findinges of increased number of branches under drip fertigation were also reported by Meenakshi and Vadivel (2003) and Muralikrishnasamy *et al.,* 2006).

The highest number of fruits per plant, length of the fruit, grith of the fruit and yield were observed under 100 per cent water soluble fertilizer along with bio stimulants fertigation treatment (T_9) in both the season (Table 4). Moreover, higher production of drymatter production under drip fertigation might have paved way for increased production of photosynthates, which ultimately resulted in increased production of chillies. This result is in accordance with the findings of Tumbare and Nikam (2004) who reported a positive response with the production of more chillies per plant under drip fertigation. In the present study, the influence of fertigation on growth attributes and physiological characteristics are reflected on the higher yield and related characters. Similar results were also reported by Veeranna *et al.,* (2000) in chilli and Kavitha (2005) in tomato.

The increase in number of fruits per plant due to liquid biofertilizers could be attributed to the higher nutrient uptake by inoculated roots and in improved water status of the plant, which in turn could be the main factors enhancing crop growth and yield (Okon, 1994). As stated in the results of present study. Bashan and Levanony (1991) also obtained highest yield with *Azospirillum* inoculation. Hence, *Azospirillum* inoculation could be considered as a substitute for partial nitrogen. Thus, increased growth and yield of crop plants due to the inoculation of *Azospirillum* have been reported by several workers since from its discovery to till date (Dobbelaere *et al.,* 2001 and Santa *et al.,* 2004).

The dry pod yield per plant and dry pod yield per hectare were observed under 100 per cent water soluble fertilizer along with bio stimulants (T_9) significantly higher in both *kharif* (185.25 g plant^{-1} and 4.05 t/ha) and *summer* season (175.25 g plant^{-1} and 3.91 t/ha) (Table 5). The pronounced increase in yield with increased dose of panchakavya might be due to sustained availability of N throughout the growing phase and also due to enhanced carbohydrate synthesis and effective translocation of photosynthates to the developing sink *i.e.,* fruit. The proportion and activity of beneficial microbes would have been at the higher rate in panchakavya which helps in synthesis of growth promoting substances might have increased the yield in this experiment. This was supported by the works Ganesh (2004) in paprika and Ramaya (2005) in annual moringa. Humic acid might have caused consistent regulation of nutrient availability during fruit development, when the uptake of nutrients is more important. Balanced nutrition of organic and inorganic nutrients maintains optimum ratio between the nutrients, which is of considerable importance in improving the yield (Muscolo *et al.,* 1993). From the investigation, it may be concluded that among the treatments, T_9 (T_α + ...) was found better with regard to increase in crop growth and yield.

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Table 1 : Effect of drip fertigation on plant height (cm) at different crop growth stages of chilli cv. KKM-1.

Treatments	Kharif				Summer			
	60 DAT	90 DAT	120 DAT	180 DAT	60 DAT	90 DAT	120 DAT	180 DAT
T_1	41.16	50.95	58.88	63.63	35.87	47.54	56.24	61.62
T_2	41.48	52.17	61.50	66.65	37.58	50.11	58.82	64.04
T_3	45.27	55.31	64.93	70.11	40.07	52.78	62.30	69.46
T_4	43.06	54.84	63.12	68.51	38.63	51.17	61.18	67.89
T_5	47.89	59.97	70.08	76.47	43.11	55.59	65.57	72.86
T_6	47.81	58.72	68.84	73.16	41.43	54.51	64.14	70.30
T_7	48.05	60.50	70.66	76.77	45.02	57.14	67.04	75.69
T_8	47.53	58.86	68.67	74.92	44.96	56.54	66.04	75.69
T_9	48.31	62.41	72.59	78.01	45.80	57.84	68.19	77.10
SEd	**0.641**	**0.851**	**1.008**	**1.088**	**0.773**	**0.761**	**0.863**	**1.121**
CD (P=0.05)	**1.359**	**1.804**	**2.137**	**2.307**	**1.640**	**1.613**	**1.829**	**2.376**

Table 2 : Effect of drip fertigation on number of primary branches at different crop growth stages of chilli cv. KKM-1.

Treatments	Kharif				Summer			
	60 DAT	90 DAT	120 DAT	180 DAT	60 DAT	90 DAT	120 DAT	180 DAT
T_1	3.53	4.41	5.23	5.75	2.89	4.22	5.07	5.57
T_2	3.61	4.59	5.51	6.03	3.31	4.59	5.38	5.89
T_3	4.54	5.45	6.35	6.93	3.57	4.84	5.75	6.34
T_4	4.40	5.33	6.23	6.83	3.36	4.65	5.58	6.19
T_5	4.95	6.07	6.98	7.57	3.99	5.36	6.31	6.85
T_6	5.03	6.13	7.03	7.65	3.75	5.11	6.05	6.70
T_7	5.41	6.38	7.29	7.89	4.83	6.17	7.12	7.84
T_8	5.24	6.23	7.08	7.73	4.28	5.79	6.77	7.40
T_9	5.50	6.46	7.39	8.04	5.06	6.39	7.35	7.92
SEd	**0.156**	**0.166**	**0.168**	**0.178**	**0.155**	**0.161**	**0.171**	**0.180**
CD (P=0.05)	**0.334**	**0.352**	**0.358**	**0.378**	**0.330**	**0.343**	**0.363**	**0.383**

Table 3 : Effect of drip fertigation on number of secondary branches at different crop growth stages of chilli cv. KKM-1.

Treatments	Kharif				Summer			
	60 DAT	90 DAT	120 DAT	180 DAT	60 DAT	90 DAT	120 DAT	180 DAT
T_1	9.86	13.39	15.82	16.48	8.92	12.59	14.89	15.73
T_2	10.34	14.43	16.29	16.88	9.67	13.15	15.62	16.63
T_3	10.51	14.91	17.20	18.33	10.05	14.33	16.84	17.70
T_4	10.38	14.69	16.91	18.02	9.93	13.52	15.78	16.82
T_5	11.22	15.51	18.12	20.18	10.74	14.84	17.47	18.35
T_6	10.84	15.33	17.91	19.95	10.51	14.10	16.56	17.44
T_7	11.77	16.22	19.05	21.04	11.49	15.47	18.44	19.33
T_8	11.45	15.99	18.97	20.71	10.96	14.46	16.91	17.86
T_9	12.03	16.38	19.22	21.85	11.84	15.92	18.62	19.62
SEd	**0.157**	**0.207**	**0.268**	**0.412**	**0.198**	**0.231**	**0.271**	**0.271**
CD (P=0.05)	**0.333**	**0.439**	**0.569**	**0.873**	**0.420**	**0.491**	**0.574**	**0.576**

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Table 4 : Effect of drip fertigation on number of Flowers/plant, no. of fruits/plant, fruit length (cm) and fruit girth (cm) of chilli cv. KKM-1.

Treatments	No. of flowers/ plant		No. of fruits/plant		Fruit length (cm)		Fruit girth (cm)	
	Kharif	*Summer*	*Kharif*	*Summer*	*Kharif*	*Summer*	*Kharif*	*Summer*
T_1	231.85	218.24	117.64	103.00	8.68	8.41	2.41	2.27
T_2	240.11	224.53	137.58	119.04	8.96	8.73	2.57	2.35
T_3	253.80	249.31	163.66	152.71	9.52	9.39	2.74	2.72
T_4	249.63	237.87	155.08	137.25	9.34	9.16	2.59	2.56
T_5	295.92	272.25	212.65	190.56	10.11	9.27	2.91	3.04
T_6	291.73	259.73	206.48	177.37	9.95	9.82	2.82	2.92
T_7	317.49	306.44	237.56	228.41	11.07	10.79	3.09	3.14
T_8	305.26	284.98	225.77	205.67	10.89	10.41	2.36	3.29
T_9	338.14	314.56	260.11	243.29	11.31	11.02	3.36	3.29
SEd	**8.130**	**7.623**	**10.56**	**10.50**	**0.204**	**0.194**	**0.062**	**0.077**
CD (P=0.05)	**17.235**	**16.162**	**22.39**	**22.26**	**0.433**	**0.411**	**0.132**	**0.163**

Table 5 : Effect of drip fertigation on fresh fruit yield/plant, dry pod yield/plant and dry pod yield/ha of chilli cv. KKM-1.

Treatments	Fresh fruit yield (g plant^{-1})		Dry pod yield (g plant^{-1})		Dry pod yield (tonnes/ha)	
	Kharif	*Summer*	*Kharif*	*Summer*	*Kharif*	*Summer*
T_1	349.08	326.93	84.50	76.60	2.26	2.12
T_2	360.55	344.93	88.71	82.53	2.41	2.33
T_3	401.19	380.48	103.93	98.27	3.05	2.88
T_4	393.23	374.95	96.86	91.52	2.79	2.64
T_5	438.57	410.24	123.74	111.83	3.56	3.45
T_6	419.25	395.52	115.28	10.6.39	3.35	3.32
T_7	464.36	437.59	142.39	131.56	3.84	3.77
T_8	439.47	415.65	128.56	116.87	3.61	3.47
T_9	510.24	462.47	185.25	174.25	4.05	3.91
SEd	**10.926**	**9.294**	**6.783**	**6.392**	**0.139**	**0.137**
CD (P=0.05)	**23.164**	**19.703**	**14.380**	**13.552**	**0.295**	**0.291**

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Changes in Soil Carbon Pool under the Influence of Long Term Fertilization Cum Intensive Cropping in an Inceptisol

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The present study was undertaken in the on-going Long Term Fertilizer Experiment since 1972 at Tamil Nadu Agricultural University, Coimbatore, India with continuous application of fertilizers and finger millet-maize cropping sequence in a Vertic Ustropept. The active carbon pools *viz*, water soluble carbon and water soluble carbohydrates were significantly influenced by the treatments. The higher amount of active carbon pools was recorded with the 100% NPK + FYM application. Fertilizer application also influenced the amount of active carbon pools to a considerable level. Continuous cropping over the years showed a build up in organic C level with all the treatments. High fertilizer input level (150% NPK) and integrated use of FYM and fertilizers recorded higher amount of organic C than control.

Keywords : Continuous application of fertilizers, Intensive cropping, Soil organic matter, Active carbon pools.

INTRODUCTION

Soil organic matter, its quality and structure, influences all matter fluxes both in soil and between soil, plants, water and atmosphere system. Understanding long-term SOC changes in various ecosystems is of importance because SOC directly affects soil quality (Bandaranayake *et al.*, 2003) through its effect on specific soil functions. These functions include serving as a medium for the growth of plant roots and root-symbionts, regulating the flow of water, air and nutrients, partitioning rainfall into plant-available, ground and surface-water, serving as a repository for atmospheric carbon, and mitigating the impacts of pollutants on human and ecosystem health. OM affects these functions by causing or mediating changes in soil properties and processes which are related to soil physical integrity, soil fertility and productivity and environmental quality.

Soil microbial biomass plays a major role as a catalyst in the decomposition and transformation of soil organic matter. Upon decomposition, about one third of the C derived from plant residues is still present in the soil after one year and decomposes slowly. Plant C is sequestered in the soil through decomposition and conversion into soil organic C in aggregates. As a result, addition of C and N from plant residues may improve soil quality and productivity by improving microbial activities, N mineralization and aggregation and increasing C and N storage in soil. Hence, the present investigation was contemplated in the on going Long Term Fertilizer Experiment since 1972, located in Tamil Nadu Agricultural University, Coimbatore, India to monitor changes in the turnover rate of soil organic matter after 29th cropping cycle.

MATERIAL AND METHODS

The investigation was carried out in the on-going long term fertilizer experiment since 1972 for studying the effects of various fertilizer treatments on a fixed cropping rotation of finger millet-maize in fine montmorillonitic and Vertic Ustropepts. The climate was semi-arid tropical with a dry season from January to June and wet season up to December. The experiment consisted of 10 treatments and was laid out in a randomized block design with four replications. The ten treatments replicated four times are (T_1) 50% NPK, (T_2) 100% NPK, (T_3) 150% NPK, (T_4) 100% NPK + Hand Weeding (HW), (T_5) 100% NPK + $ZnSO_4$ (Maize alone), (T_6) 100% NP, (T_7) 100% N, (T_8) 100% NPK + FYM (Fingermillet alone@ 10 t ha^{-1}), (T_9) 100% NPK (-S) and (T_{10}) Control. The optimal amounts of N, P_2O_5 and K_2O (100% NPK) are 90 : 45 : 17.5 and 135 : 67.5 : 35 Kg NPK ha^{-1} for fingermillet and maize, respectively. The fertilizers used were urea, single superphosphate, diammonium phosphate (T_9 only), muriate of potash and zinc sulphate. The soil of the experimental site was sandy clay loam in texture with a pH of 8.2 and EC of 0.20 dSm^{-1}. The organic C content was 0.30% and the soil was low (80 mg Kg^{-1}), medium (4.9 mg Kg^{-1}) and high (362 mg Kg^{-1}) status in available N,P and K respectively. The water soluble organic carbon and carbohydrates were determined as per McGill *et al.*, (1986) and Brink *et al.*, (1960) and Biomass C and N by the fumigation - incubation technique (FIC) - Jankinson and Powlson (1976) in soil after harvest.

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RESULTS AND DISCUSSION

The results revealed that continuous adoption of all treatments to the high intensive crop system tended to build up soil organic matter over years. This was ture even with the unmanured contol when compared to the initial level of 0.30 per cent of organic carbon during 1972 (Fig. 1). Organic carbon in soil increased with increasing levels of fertilizer NPK evidently because of the return of crop residues through incorporation of stubbles in increased amounts from well grown crops at high level of fertilizer inputs (Bharadwaj and Omanwar, 1994). The continuous application of fertilizers incorporated with FYM recorded the highest organic carbon content (0.70 mg Kg^{-1}), whereas the lowest (0.42 mg Kg^{-1}) was recorded in the control. The increase could be attributed to addition of organic matter and also due to better root growth, more plant residues after harvest of the crop indirect influence on the physico-chemical characteristics of the soil. Continuous addition of 10 t FYM per ha (to finger millet alone) with recommended dose of NPK increased the organic C content by 133 per cent, the increase were 93 per cent and 40 per cent in 100 per cent NPK and control, respectively. The application of 150% of recommended NPK recorded 123 per cent increase in soil organic carbon content after years. Application of N alone (100% N) increased organic carbon level in soil by only 10% whereas application on N and P (100% NP) increased it by 22% over control. The K application along with N and P (100% NPK) did not increase organic carbon level in soil. The organic carbon content exhibited and increasing trend with increase in the number of crops grown which could be due to cumulative addition of left over residues (Tolanur and Badanur 2003).

Active Carbon Pools : The highest water soluble organic carbon and carbohydrates were observed with continuous addition of FYM in association with 100% NPK fertilizers whereas the lowest the content was in the control (Table 1). The higher amount of water soluble organic carbon in the 100% NPK + FYM treatment may be due to its origin from soil biomass and root exudates and lysates, and its presence in soil solution or weakly adsorbed by soil minerals and humic macromolecules. Lienweber *et al.*, (1995) reported that long-term fertilization with NPK + FYM resulted in larger mean concentration of hot water-extracted C and N than soil management without fertilization. Integrating FYM + NPK increased the biomass C and N by 90 and 40 per cent respectively over control. There was a slight reduction in biomass C with S free ferilizer application as compared to that of optimal NPK, but the difference was not significant.

CONCLUSION

The present study implied that continuous application of 100% NPK + FYM 10 t FYM ha^{-1} under continuous cropping increased the organic C and active carbon pools thereby could sustain the crop and soil productivity.

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Table 1 : Effect of treatments on active carbon pools in soil after Maize (mg Kg^{-1}).

Treatments	Water soluble Organic carbon	Water soluble carbohydrate	Biomass C (mg Kg^{-1})	Biomass N (mg Kg^{-1})
T_1 - 50% NPK (RD)	74.0	540	264	40
T_2 - 100% NPK (RD)	84.0	552	280	48
T_3 - 150% NPK (RD)	105.5	573	312	58
T_4 - 100% NPK + HW	84.8	532	272	44
T_5 - 100% NPK + $ZnSO_4$ (Maize)	86.7	557	286	40
T_6 - 100% NP	77.7	536	288	36
T_7 - 100% N alone	70.4	529	248	32
T_8 - 100% NPK + FYM (Finger millet)	128.6	651	344	60
T_9 - 100% NPK (S free)	89.3	547	286	36
T_{10} - Control	55.1	435	230	30
SEd	5.20	7.38	9.88	3.74
CD (P = 0.05)	10.66	15.14	19.8	7.6

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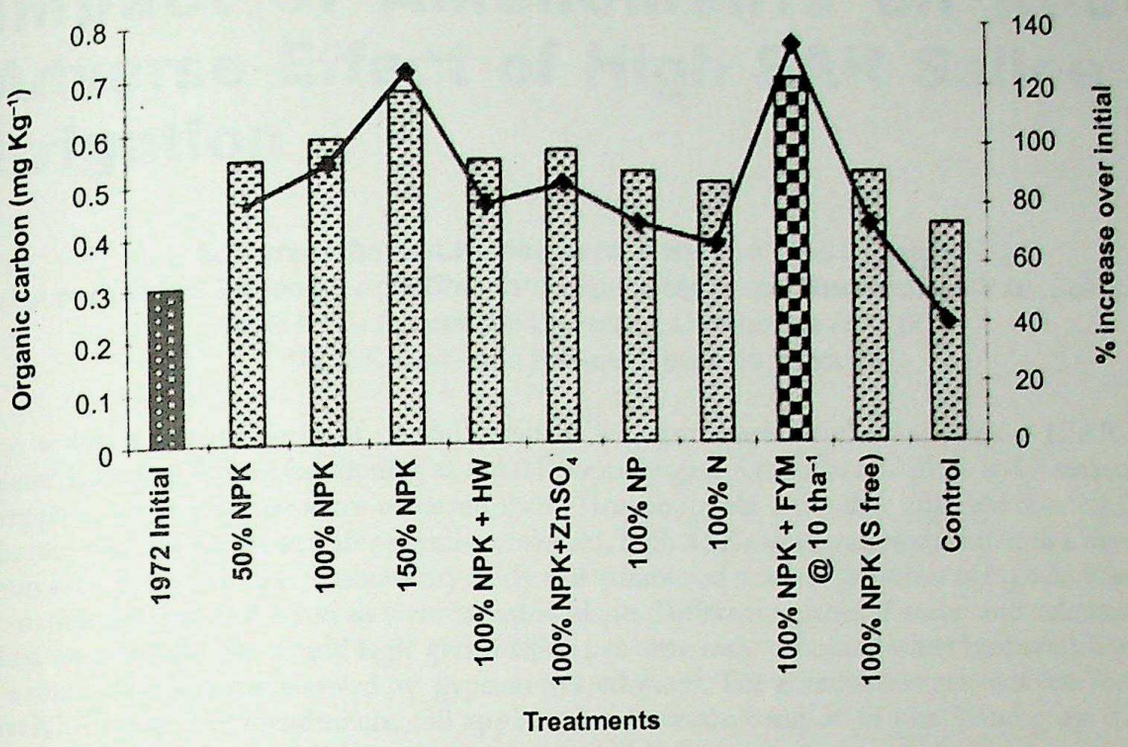

Fig. 1. Changes in Organic Carbon content as influenced by treatments and per cent increase over initial status after 29th Cropping Cycle.

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Impact of Amendments on Reducing Adverse Effect of High SAR Saline Water Irrigation

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Irrigation water is one of the most critical resource for agricultural production. Under ICAR- All India Coordinated Research Project functioning at Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli, water samples were collected from Tiruchirappalli and other adjacent districts for ground water characterization. Of these water samples collected, high SAR saline waters are found in Udiyur block of Kankeyam area, Erode district. A laboratory study was conducted to study the effect of high SAR saline water on germination per cent and biomass yield in cotton plants. Different sources of water and amendments were used. Maximum biomass yield and high germination per cent were recorded when best available water was used as a source of irrigation followed by gypsum treated water. The germination per cent was 90.0 and 82.5 respectively. Among the amendments, soil application of vermin compost (5 t ha^{-1}) and press mud (t ha^{-1}) registered higher germination per cent.

Keywords : High SAR saline water, Gypsum, Cotton plants, Biomass yield.

INTRODUCTION

Water is an important input in production process and because of its paramount importance in life it has got special value. Groundwater surveys indicate that poor quality waters being utilized in different states range between 32 - 84 per cent of the ground water development. Development of salinity, sodicity and toxicity problems in soils due to poor quality irrigation water not only reduce crop productivity, but also deteriorate the quality of produce and limit the choice of crops. Many times the effect become so severe that lands eventually go out of cultivation. The Electrical Conductivity(EC), Sodium Absorption Ratio (SAR) and Residual Sodium Carbonate (RSC) values for high SAR saline water are > 4.0dSm^{-1}, >10.0 and < 2.5 m.eq/ lit. respectively (AICRP- Annual report-1991). Irrigation with high SAR saline water has got both salinity and sodicity hazard. Saline water irrigation adversely affect plant growth primarily through the impact of excessive salts in soil solution. It retards the absorption of essential plant nutrients. Water containing high proportion of sodium carbonate increases the exchangeable sodium in the field which result in deflocculation of clay which decreases soil permeability and aeration and ultimately the crop growth. It has negative influence on germination percentage, root growth, absorption of water and nutrients.The adverse effect of both salinity and sodicity in high SAR saline water can be mitigated by the use of amendments which contains calcium. Hence, laboratory study was conducted to study the effect of different amendments on germination per cent and biomass yield of cotton plants.

MATERIAL AND METHODS

A laboratory study was conducted at Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli, in factorial completely randomized design with cotton variety RAHS-14 as a test crop. Soil was collected from the field, processed and filled in paper cups @ 300 g per cup and the soil was treated with different amendments *ie.* Gypsum @ 2.5 t / ha, 5.0 t / ha, vermicompost @ 5.0 t / ha and pressmud @ 4.0 t / ha. Different sources of water *viz.*, high SAR saline water , best available water and gypsum treated water were used for watering the plant. High SAR saline was collected from bore well located in the farmers field of Kankeyam area. The experiment was replicated four times and two seeds were sown in each cup (2 plants/ replication). Soil moisture content was maintained at field capacity level. Cotton plants were uprooted at 30th day after sowing and observations were made on biomass yield and shoot and root length. The uprooted plant samples were processed and analysed for sodium content. The experimental soil was non saline (EC - 0.4 dSm^{-1}), neutral in pH (7.2), low in available nitrogen (210 Kg ha^{-1}), medium in available phosphorus (18 Kg ha^{-1}) and high in potassium (400 Kg ha^{-1}) availability. The quality of water used for irrigation is given below.

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Water quality

Particulars	EC (dSm^{-1})	RSC (meq/l)	SAR
High SAR Saline water	5	-	14.0
Gypsum treated water	5.5	-	9.0
* Best available water	1.5	3.0	6.7

*The Electrical conductivity and Sodium Asorption Ratio of the water available in the study area were below 2 (dSm^{-1}) and 10 respectively. Hence it is denoted as best available water. This water is best among the rest of the water available in the study area.

RESULTS AND DISCUSSION

Germination per cent : The effect of different water sources and amendments on cotton seed germination is given in Table 1. The results reveals that the germination per cent was high when irrigated with best available water (90 %) followed by gypsum treated water (82.5%) and high SAR saline water (65%). Of the amendments tried, organics recorded highest germination followed by gypsum amendment. When organic amendments were used along with best available water as a source of irrigation, hundred per cent germination was obtained. In no amendment with high SAR saline water irrigation the germination was poor to the tune of 50 per cent. Ayers and Westcot (1985) reported that cotton is sensitive during germination and early growth stage. The reduction in germination per cent from 92.3 to 66 with increase in soil ESP from 19 to 35 has also been reported by Acharya *et al.* (2004) in cotton.

Application of gypsum and organics as soil or water amendments is recommended to offset the deteriorating effect of high SAR saline water. The ameliorative effect of organics might be the reason for higher germination per cent and better establishment of crop during early stage. The amelioration is due to alteration in the ionic ratio of the water used for irrigation.

Biomass yield : The effect of different sources of irrigation water and amendments on biomass yield is given in Table 2. Maximum biomass yield of 1.05 g was obtained in best available water irrigation followed by gypsum treated water and high SAR saline water. Among the amendments organics registered higher biomass (1.1 and 1.07g) followed by gypsum application @ 5 and 2.5 t/ha (0.85 and 0.92 g). When vermicompost @ 5 t / ha was used along with best available water source, the biomass yield was higher (1.21g) than control (0.50 g) wherein the per cent increase in biomass was 140. Gupta and Yadav (1986) were noted such yield reduction in various crops for saline water irrigation. Similar adverse effect of higher salinity water on yield of mungbean was also reported by Tripathi and Praveen Kumar (1999). In experiments conducted at Agra under All India Coordinated Project on pearl millet - wheat irrigated with saline water with varying SAR (10- 40) , gypsum application @ 25 % GR improved the average yield of pearl millet by 5 to 23. Manchanda *et al.* (1984) obtained yield increase from 0.06 t/ha to 2.67 t/ha in wheat crop for the gypsum application @ 25 % GR under alkali water irrigation. Choudary *et al.* (2003) and Minhas *et al.* (1995) also reported the synergistic effect of gypsum and FYM in improving sugar and wheat yield respectively.

The effect of gypsum on biomass yield is due to its mitigating effect of Ca on sodium. Organic manure has a beneficial acidifying effect on sodicity through the action of organic acids formed during its breakdown and also through mobilization of inherent soil Ca^{2+} from $CaCO_3$ and other minerals by organic acids. From the result it is concluded that application of gypsum or any organic amendments is essential for achieving higher germination per cent, better establishment and yield under high SAR saline water irrigation and several management practices should be considered in integrated manner to mitigate the adverse effect of poor quality water.

Shoot and root length : The data on shoot length indicated the adverse effect of high SAR saline water (Table 3). The shoot length was maximum (11.4 cm) in vermicompost treatment followed by pressmud and gypsum application which were found to be on par with each other. Among the water source, best available water and gypsum treated water were on par as against the lowest value of 9.09 cm in high SAR saline water.

Gypsum application @ 2.5 t / ha and 5 t/ha recorded higher root length values of 9.73 and 9.5 cm respectively while it was 7.99 cm in no amendment (Table 4). With respect to water source, the corresponding root length were 6.97, 10.27 and 9.36 cm in high SAR saline water, best available water and gypsum treated water. The result also indicated the significant interaction of water source with amendments. The highest shoot and root length of 11.7 and 11.35 cm were recorded in best available water with gypsum application followed by gypsum treated water. When high SAR saline water was used with no amendment, the shoot and root growth was found to be poor (6.3 and 4.6 cm). The reduction was 85 and 146 per cent respectively. Such significant reduction in plant height was recorded by Tripathi and Praveen Kumar (1999) in mungbean when poor quality water was used for irrigation.

The shoot and root growth was good when the poor quality water was treated with gypsum along with soil application of gypsum and organic amendments. This might be due to the presence of Ca ion and their mitigating effect on salinity and sodicity hazards (*i.e.*, altering the ionic ratio) which leads to better soil aggregation, permeability and nutrient uptake.

Sodium content : Sodium content of 3.15 per cent was recorded in no amendment as against 2.59 per cent in pressmud application which was on par with vermicompost. Among the different water sources tried, high SAR saline water recorded the highest sodium content (3.76 %) when compared to other water sources. As regards to interaction of different water sources with amendments, highest content of 3.88 per cent was recorded in high SAR saline water with no amendment and lowest of 1.4 per cent in best available water with vermicompost treatment. Misra (2001) reported the increased sodium and decreased potassium content in chickpea when sodicity level was increased. With respect to interaction of

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Table 1 : Effect of high SAR saline water on germination per cent.

Treatment	High SAR Saline water	Best available water	Gypsum treated water	Mean
No amendment	50.0	75.0	62.5	62.5
Gypsum @ 2.5 t ha^{-1}	62.5	87.5	87.5	79.2
Gypsum @ 5 t ha^{-1}	62.5	87.5	87.5	79.2
Vermicompost 5 t ha^{-1}	75.0	100	87.5	87.5
Pressmud 4 t ha^{-1}	75.0	100	87.5	87.5
Mean	65.0	90.0	82.5	

Table 2 : Effect of high SAR saline water on biomass yield (dry weight-g /cup).

Treatment	High SAR Saline water	Best available water	Gypsum treated water	Mean
No amendment	0.50	0.85	0.81	0.72
Gypsum @ 2.5 t ha^{-1}	0.79	0.93	0.84	0.85
Gypsum @ 5 t ha^{-1}	0.81	1.08	0.90	0.92
Vermicompost 5 t ha^{-1}	1.06	1.21	1.03	1.10
Pressmud 4 t ha^{-1}	1.02	1.19	1.02	1.07
Mean	0.84	1.05	0.92	
	A	I	A x I	I x A
CD (0.05)	0.02	0.017	0.39	0.42

Table 3 : Effect of high SAR saline water on shoot length (cm).

Treatment	High SAR Saline water	Best available water	Gypsum treated water	Mean
No amendment	6.38	9.70	7.98	7.99
Gypsum @ 2.5 t ha^{-1}	9.33	11.68	11.15	10.72
Gypsum @ 5 t ha^{-1}	9.25	11.70	11.60	10.85
Vermicompost 5 t ha^{-1}	10.38	11.55	11.50	11.14
Pressmud 4 t ha^{-1}	10.23	10.95	11.65	10.94
Mean	9.09	11.12	10.78	
	A	I	A x I	I x A
CD (0.05)	0.29	0.35	0.57	0.51

Table 4 : Effect of high SAR saline water on root length (cm).

Treatment	High SAR Saline water	Best available water	Gypsum treated water	Mean
No amendment	4.60	8.43	7.00	6.68
Gypsum @ 2.5 t ha^{-1}	7.87	10.80	10.10	9.59
Gypsum @ 5 t ha^{-1}	7.70	11.35	10.13	9.73
Vermicompost 5 t ha^{-1}	7.40	10.20	9.80	9.13
Pressmud 4 t ha^{-1}	7.30	10.58	9.75	9.21
Mean	6.97	10.27	9.36	
	A	I	A x I	I x A
CD (0.05)	0.27	0.37	0.56	0.47

amendments with different water sources, the sodium content in the plant was found to be lower when pressmud was used as amendments along with high SAR saline water followed by vermicompost and gypsum treatment.

In view of the above study, it can be concluded that application of gypsum and organics as soil or water amendments is essential to offset the deteriorating effect of high SAR saline water on soil properties and plant growth and yield.

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Table 5 : Effect of high SAR saline water on sodium content (%).

Treatment	High SAR Saline water	Best available water	Gypsum treated water	Mean
No amendment	3.88	2.25	3.33	3.15
Gypsum @ 2.5 t ha^{-1}	3.79	1.82	2.89	2.83
Gypsum @ 5 t ha^{-1}	3.71	1.48	2.84	2.68
Vermicompost 5 t ha^{-1}	3.76	1.40	2.69	2.61
Pressmud 4 t ha^{-1}	3.67	1.46	2.66	2.59
Mean	3.76	1.68	2.88	
	A	I	A x I	I x A
CD (0.05)	0.034	0.038	0.078	0.086

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Effect of Distillery Effluent on the Seed Germination and Seedling Growth of *Sorghum bicolor* (L.) Moench

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Distillery effluent of Pravara distillery was physico-chemically analysed and it's effect on seed germination and seedling growth of *Sorghum bicolor* was investigated. The effluent had beneficial effects at lower concentrations (5%, 10% and 15%) but lethal effects at higher concentrations (25%, 50%, 75% and 100%). Increase in effluent concentration showed proportional decrease in seed germination and seedling development.

Keywords : Distillery effluent, Seed germination, Root length, Shoot length, Vigor index.

INTRODUCTION

Effluents discharged from the industries are commonly used in agriculture. The beneficial and lethal effects of effluents at different concentrations on seed germination and seedling development was assessed by several workers (Sahai *et al.* 1983 a & b; Rajaram and Janardhanan, 1988; Behera and Mishra, 1982; Singh *et al.,* 1985; Somasekhar *et al.,* 1992; Subramani *et al*, 1995 and Reddy and Borse, 2001). They recommended that the effluent could be used as a liquid fertilizer for crops after dilution.

In this paper effect of effluent discharged from Pravara distillery on the seed germination and seedling growth of *Sorghum bicolor* is reported.

Pravara distillery located at Pravaranagar of Ahmednagar district in Maharashtra state (Map). was established in 1970. Nearly 60,000 liters of alcohol is produced daily and 7.5 - 8 lakh liters of effluent per day is discharged. The effluent released is used for irrigation purpose by farmers.

MATERIAL AND METHODS

Untreated samples of the effluent were collected from the outlet of the Pravara distillery. The temperature and pH values of the effluent were noted at the collection site and samples were brought to the laboratory in plastic containers. The physico-chemical analysis of the effluent (Table 1) was done following the APHA (1989) methods. Different concentrations of the effluent *viz.*: 5%, 10%, 15%, 25%, 50%, 75% and 100% were prepared by proportionately adding distilled water. Healthy seeds were collected from Mahatma Phule Agricultural University, Rahuri for experimental studies. Seeds were first surface sterilized with 1% $HgCl_2$ for about 2 minutes and washed twice in sterile distilled water. Twenty seeds were soaked in each concentration for 2 hours and then were evenly placed on a blotter paper in separate Petri plates and irrigated with corresponding concentration of the effluent. The seeds were kept for germination at 28 ± 2°C. A set of duplicates was maintained. The sets treated with distilled water served as control. On seventh day, number of seeds germinated was noted. Emergence of radicle from seed was taken as a criterion for considering the seed as germinated.

Seed germination percentage, average shoot length, root length and vigour index were calculated. Vigour index was calculated following Abdul Baki and Anderson (1973). Ten seedlings from each plate were randomly taken for estimating protein and sugar following Lowry, *et al.* (1951), and Nelson (1944) respectively. RNA was estimated by orcinol method given by Sawhney and Randhir Singh, (2007). The results were subjected to statistical analysis and are expressed as arithmetic means with their standard deviation. Student ' t ' test was applied to determine the differences between the control and experimental.(Table 2).

RESULTS AND DISCUSSION

The effluent is acidic, dark brown without any dissolved oxygen. Total solids, total dissolved solids, total suspended solids, BOD and COD values are higher than the ISI limits (IS: 3307, 1974) set for the discharge of industrial effluents for irrigation (Table-1).

Seed germination percentage, root length, shoot length , vigour index ,wet and dry weight, protein, sugar and RNA content was higher in the seeds treated with 5 %, 10 % and 15 % effluent concentration which decreased proportionately with an increase in the concentration from 25 to 100 % as compared with control. Thus the effluent had beneficial and

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harmful effects on seed germination and seedling development at lower and higher concentrations respectively. The effluent at 15% concentration level may be used as a liquid fertilizer since it showed maximum beneficial effect (Table-2).

Subramani *et al.*, (1995) is of the opinion that the beneficial effect of effluent at lower concentrations may be due to the presence of optimum levels of plant nutrients in it.

The present observations are similar with those of Behera and Mishra, (1982); Sahai *et al.* (1983 a & b); Singh *et al.* (1985); Sahai, and Shrivastava,.(1986); Subramani, *et al;* (1995); Kannan (2001) and Reddy and Borse (2001).

ACKNOWLEDGEMENT

The authors are thankful to the Principal, Padmashri Vikhe Patil College, Pravaranagar for encouragement and providing laboratory facilities. Thanks are due to the Director, Pravara distillery pvt. ltd., Pravaranagar for the help and cooperation.

Table 1 : Physico- Chemical characteristics of the Distillery effluent.

S.No.	Particulars	Value
1.	Colour	Dark brown.
2.	Odour	Aromatic
3.	pH	4.84
4.	Electrical conductivity	1.83 mmhos/cm.
5.	Temperature	43 °C
6.	Total Solids	64600.00
7.	Total Dissolved Solids	61800.00
8.	Total Suspended Solids	2800.00
9.	Biochemical Oxygen Demand	38000.00
10.	Chemical Oxygen Demand	76800.00
11.	Dissolved Oxygen	Nil
12.	Free CO_2	6600.00
13.	Oil & grease	100.00

All values except pH, temperature and conductivity are expressed in mg / lit.

Table 2 : Effect of effluent at different concentrations on seed germination % and seedling growth of *Sorghum bicolor*.

S.No.	Concen-tration	% age of germ-ination	Shoot length (cms)	Root length (cms)	Vigour index	Wet wt. (gms)	Dry wt. (gms)	Protein content (µg/gm)	Sugar content (µg/gm)	RNA content (µg/gm)
1.	Control	100	3.0 ± 0.3	3.6 ± 0.46	660	0.68	0.59	69.	78	96
2.	5	100	3.3 NS ± 0.36	3.8 NS ± 0.53	710	0.71	0.60	72	80	99
3.	10	100	3.4 NS ± 0.26	3.8 NS ± 0.35	720	0.71	0.59	71	80	99
4.	15	100	4.5** ± 0.36	4.9* ± 0.53	940	0.83	0.72	84	97	105
5.	25	85	2.1 NS ± 0.53	2.6* ± 0.17	399.5	0.63	0.52	62	60	90
6.	50	60	1.5** ± 0.26	2.2* ± 0.53	222	0.41	0.30	40	32	38
7.	75	20	1.2** ± 0.26	2.0** ± 0.2	64	0.29	0.19	28	18	32
8.	100	5	1.2** ± 0.9	2.0** ± 0.3	16	0.22	0.13	21	11	28

All values are expressed as means of ± SD
NS : Non Significant ; * : $p < 0.05$; ** : $p < 0.01$

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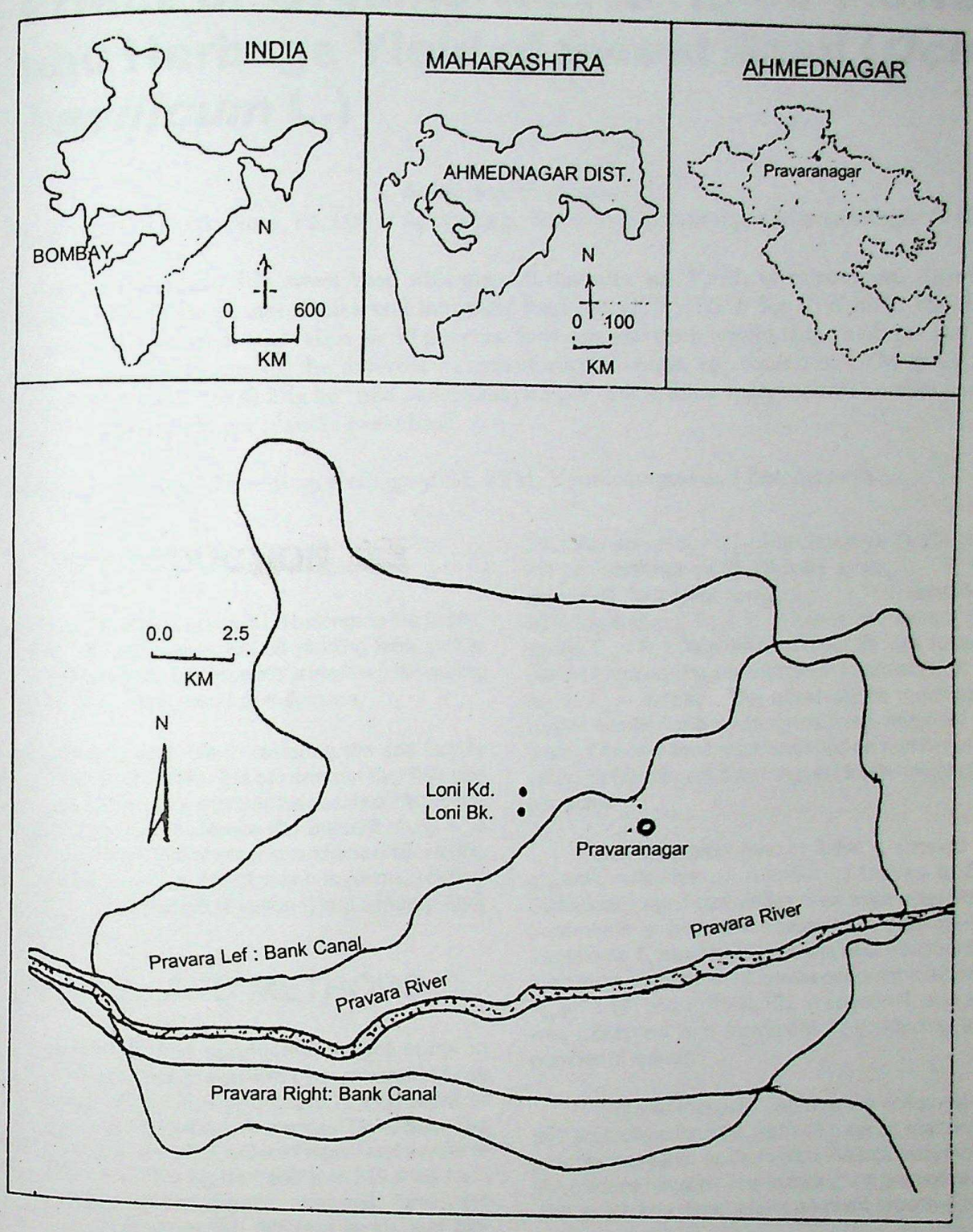
Location map of Pravara distillery



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Effect of Organic Nutrients on Flowering and Herbage Yield of Sweet Basil (*Ocimum basilicum* L.)

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A pot study was conducted in sweet basil with organic manures *viz.*, FYM, vermicompost, *Azospirillum, posphobacteria,* panchakavya, neem cake and inorganic fertilizers (120:100:100 Kg NPK ha^{-1}). The data on number of leaves produced, time taken for 50 per cent flowering and fresh weight of the herb per plant were collected and analysed. Among the different treatment combinations, application of FYM @ 25 ha^{-1} + *Azospirillum* + *Posphobacteria* @ 2Kg ha^{-1} and panchakavya 3 per cent as foliar spary recorded earlier flowering and highest herbage yield per plant in sweet basil.

Keywords : Sweet basil, Flowering, Herbage yield, FYM, Vermicompost and Panchakavya.

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) belongs to the family, Lamiaceae is an important essential oil yielding herb and is mostly used in confectionery and food industries as a flavouring agent and also in the pharmaceutical industries.

Organics play a vital role in restoring the soil fertility and stability in crop productivity. It is of common fact that use of organic manures in orchards improve the quality of harvested produce (Kunte *et al.*, 1997). Hence the present study was carried out to find out the effect of organic nutrients on flowering and herbage yield of sweet basil (*Ocimum basilicum* L.) during two seasons, July-October 2008 (Season-I) and January-April 2009 (Season-II).

MATERIAL AND METHODS

The experiment was conducted as pot study in completely randomized block design with 14 treatments in three replications during 2007-2009 at orchard, Department of Horticulture, Faculty of Agriculture, Annamalai University. The soil texture is clay loam, pH is 7.5, EC 0.67 dsm^{-1} and available N is 210.5 Kg ha^{-1}, P is 10.3 Kg ha^{-1} and K is 249.4 Kg ha^{-1}. Treatments consisted of two organic manures, farm yard manure and vermicompost at two different levels and bio-fertilizers (*Azospirillum* and *Phosphobacteria*) along with panchakavya 3 per cent and neem cake 20 per cent as foliar spray. The following are the treatments T_1 - FYM @ 12.5 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}, T_2 - FYM @ 25 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}, T_3 - Vermicompost @ 2.5 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}, T_4 - Vermicompost @ 5 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}, T_5 - T_1 + Panchakavya @ 3% foliar spray, T_6 - T_2 + Panchakavya @ 3% foliar spray, T_7 - T_3 + Panchakavya @ 3% foliar spray, T_8 - T_4 + Panchakavya @ 3% foliar spray, T_9 - T_1 + Neemcake extract @ 20% foliar spray, T_{10} - T_2 + Neemcake extract @ 20% foliar spray, T_{11} - T_3 + Neemcake extract @ 20% foliar spray, T_{12} - T_4 + Neemcake extract @ 20% foliar spray, T_{13} - Recommended dose of inorganic fertilizer (120: 100: 100 Kg ha^{-1}), T_{14} - Control. The observations recorded were plant height number of branches and fresh weight of the herb per plant. Observations were recorded on number of leaves, time taken for 50 per cent flowering and fresh weight of the herbage per plant.

The data presented in Table 1 showed the effect of organic nutrients on number of leaves in Sweet basil. Statistically significant differences were observed among the treatments in both the seasons. In season-I, among the treatments T_6 was found to be the best treatment in producing the maximum number of leaves per plant (692.48) followed by T_{13} (692.14) and T_{12} (660.12). In season-II, also a similar trend was observed with treatment T_6 producing the maximum number of leaves.

Organic manures improve the soil physical conditions and promote microbial and soil organic matter, which in turn produces organic acids, which inhibits enzymes, particularly IAA oxidase resulting in enhancing the promotive effect of auxin -IAA which has direct effect on plant growth (Leopold, 1974).

The data on time taken (days) for 50 per cent flowering was presented in Table - 2. In the season-I, the plants in the treatment T_6 recorded the least number of days (48.19) for 50 per cent flowering which was closely followed by treatment T_{13} (49.21) and T_{12} (50.14). In the season-II, the minimum number of days taken for 50 per cent flowering was recorded in the treatment T_6 (49.50) followed by treatment T_{13} (49.48) and T_{12} (50.95). The maximum number of days taken was observed in the treatment T_{14} (absolute control) in both season-

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I (55.76) and (55.83) season-II. Comparison of two seasons, the plants in season-I recorded the minimum number of days taken for producing 50 % flowering than season-II.

From the data presented in Table 3, it is known that herbage yield per plant was found to vary significantly among the treatments in season-I and season-II. The treatment T_6 recorded the highest herbage yield of 256.99 gm /plant which was significantly higher than T_{13} 249.84 gm and T_{12} 210.00 gm/plant in season-I. Similar results were observed in season-II and the same treatment T_6 registered the highest herbage yield of 239.66 gm/plant followed by T_{13} 228.84 gm/ plant and T_{12} 200.84 gm/plant. The treatment T_{14} (absolute control) recorded lowest herbage yield of 191.00 and 187.40 gm/plant respectively in both the seasons. Among the two seasons, season-I excelled season-II in producing maximum fresh weight, irrespective of the treatments.

Bio-fertilizers might have served to provide a conductive atmosphere in soil for nitrogen accumulation, besides in soil for making increased availability of nutrients, especially by solublizing the phosphorus. Moreover, the panchakavya applied as foliar might have been absorbed readily by the plants. The favourable effect of bio fertilizers in enhancing the growth and yield attributes as observed in the present study have also been reported in a number of horticultural crops. Hemalatha *et al.*, (2008) in Kalmegh and Kalyanasundaram *et al.*, (2008) in Sweetflag. Manjunatha *et al.* (2002) have reported the beneficial effect of bio-fertilizers singly and in combinations with organic and inorganic nutrients and these results which lend support to the findings of the present study.

Panchakavya has significantly increased the herbage yield due to the presence of beneficial microorganisms and nitrogen, calcium, cytokinin, glucose, minerals etc. These nutrient rich environment with hormone might have triggered rapid cell division, proliferation and speedy growth. More over foliar application of panchakavya might have resulted in speedy absorption and assimilation of varied nutrients resulting in higher vegetative growth. The favourable effect of panchakavya as enhancer of yield in combination with other organic amendments have been reported by Ponni and Arumugam Shakila (2007) in *Phyllanthus niruri*; Mohanalaxmi and Vadivel (2008) in ashwagandha and these reports are in agreement with the results of the present study.

Based on the consistent result obtained as above and taking into consideration of the favourable effects on growth attributes and herbage yield application of FYM @ 25 t ha^{-1} + *Azospirillum* + *phosphobacteria* + Panchakavya @ 3 per cent foliar spray given three times at 30, 45 and 60 DAP can be adjudged as the best treatment combination to maximize growth and herbage yield in sweet basil. In general, the growth and herbage yield content was comparatively more in crop when sweet basil was raised during season-I (July- October 2008) as compared to season-II (January -April 2009) summer season.

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Table 1 : Effect of organic nutrients on number of leaves in sweet basil (*Ocimum basilium* L.).

Treatments	Number of leaves per plant	
	Season - I*	Season - II**
T_1 - FYM @ 12.5 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}	422.56	410.25
T_2 - FYM @ 25 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}	481.06	464.25
T_3 - Vermicompost @ 2.5 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}	424.84	410.11
T_4 - Vermicompost @ 5 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}	451.72	432.18
T_5 - T_1 + Panchakavya @ 3% foliar spray	581.66	564.28
T_6 - T_2 + Panchakavya @ 3% foliar spray	692.48	680.76
T_7 - T_3 + Panchakavya @ 3% foliar spray	525.92	512.39
T_8 - T_4 + Panchakavya @ 3% foliar spray	608.60	598.42
T_9 - T_1 + Neemcake entract @ 20% foliar spray	541.76	532.14
T_{10} - T_2 + Neemcake extract @ 20% foliar spray	630.28	612.24
T_{11} - T_3 + Neemcake extract @ 20% foliar spray	512.92	498.21
T_{12} - T_4 + Neemcake extract @ 20% foliar spray	660.12	648.32
T_{13} - Recommended dose of inorganic fertilizer (120: 100: 100 Kg ha^{-1})	692.14	672.12
T_{14} - Control	373.88	351.72
S.Ed	**10.25**	**3.25**
CD at 5 %	**20.50**	**6.50**

* July - October 2008 (Monsoon) ** January - April 2009 (Summer)

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Table 2 : **Effect of organic nutrients on time taken for 50 per cent flowering in sweet basil (*Ocimum basilium* L.).**

Treatments	Time taken for 50 per cent flowering	
	Season - I*	Season - II**
T_1 - FYM @ 12.5 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}	54.84	55.20
T_2 - FYM @ 25 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}	53.94	54.38
T_3 - Vermicompost @ 2.5 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}	55.12	55.98
T_4 - Vermicompost @ 5 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}	54.23	55.60
T_5 - T_1 + Panchakavya @ 3% foliar spray	52.57	52.86
T_6 - T_2 + Panchakavya @ 3% foliar spray	48.19	49.50
T_7 - T_3 + Panchakavya @ 3% foliar spray	53.28	52.24
T_8 - T_4 + Panchakavya @ 3% foliar spray	52.00	51.82
T_9 - T_1 + Neemcake entract @ 20% foliar spray	51.15	53.24
T_{10} - T_2 + Neemcake extract @ 20% foliar spray	50.67	51.15
T_{11} - T_3 + Neemcake extract @ 20% foliar spray	51.75	53.96
T_{12} - T_4 + Neemcake extract @ 20% foliar spray	50.14	50.95
T_{13} - Recommended dose of inorganic fertilizer (120: 100: 100 Kg ha^{-1})	49.21	49.48
T_{14} - Control	55.76	55.83
S.Ed	**0.28**	**0.23**
CD at 5 %	**0.57**	**0.46**

* July - October 2008 (Monsoon) ** January - April 2009 (Summer)

Table 3 : **Effect of organic nutrients on fresh weight of the herb in sweet basil (*Ocimum basilium* L.).**

Treatments	Fresh weight of the herbage per plant (gm)	
	Season - I*	Season - II**
T_1 - FYM @ 12.5 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}	222.13	202.20
T_2 - FYM @ 25 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}	239.06	218.80
T_3 - Vermicompost @ 2.5 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}	221.13	214.93
T_4 - Vermicompost @ 5 t ha^{-1} + *Azospirillum* and *Phosphobacteria* @ 2 Kg ha^{-1}	237.13	210.11
T_5 - T_1 + Panchakavya @ 3% foliar spray	242.69	215.06
T_6 - T_2 + Panchakavya @ 3% foliar spray	256.99	239.66
T_7 - T_3 + Panchakavya @ 3% foliar spray	238.46	215.33
T_8 - T_4 + Panchakavya @ 3% foliar spray	239.19	219.13
T_9 - T_1 + Neemcake entract @ 20% foliar spray	229.06	221.93
T_{10} - T_2 + Neemcake extract @ 20% foliar spray	246.13	217.40
T_{11} - T_3 + Neemcake extract @ 20% foliar spray	220.66	209.60
T_{12} - T_4 + Neemcake extract @ 20% foliar spray	210.00	200.84
T_{13} - Recommended dose of inorganic fertilizer (120: 100: 100 Kg ha^{-1})	249.84	228.84
T_{14} - Control	191.00	187.40
S.Ed	**3.02**	**4.15**
CD at 5 %	**6.04**	**8.30**

* July - October 2008 (Monsoon) ** January - April 2009 (Summer)

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Field Evaluation of Some Insecticides and Biopesticides Against Red Rust Thrips (*Cheatanophothrips signipennis*) in Banana

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Banana red rust thrips *Cheatanophothrips signipennis* has become an important pest of banana causing reddish brown or black discoloration and cracks on fruit surface affecting its marketable quality. In view of this different chemicals and biopesticides were tried for the control of this pest and their economics was also worked out. The trial was conducted in randomized block design with three replications and nine treatments for three seasons during 2003-2007. Two sprays, first at flag leaf stage and second spray or just shooting stage and second spray seven days after first spray were given. In this study all the chemical and biopesticidal treatments were found significantly superior over the unteated control. The treatment acetamiprid 20 WP 0.0025% was found most effective for the control of red rust thrips damage (18.67%) on banana fruits. However, it was on par with monocrotophos 0.05% (20.32%), deltamethrin 0.001% (20.58%), acephate 0.1125% (20.26%), *Verticillium lecanii* (@ 2 x 10^8 cfu/ml) 3 g/lit (21.11 %) and dimethoate 0.03% (21.26%). Biopesticides namely *Verticillium lecanii* (@ 2 x 10^8 cfu/ml) 3 g/lit and NSKE 5%. also showed significant reduction (21.11% and 22.90%, respectively) in the damage of rust thrips and therefore can also be used as an ecosafe measure.
As regards the yield, the treatments did not differ significantly. However monetary returns of Rs. 221191. 70/ha due to treatment acetamiprid were significantly highest which was also has highest cost benefit ratio 1.46.

Keywords : Banana, Flower Thrips *Cheatanophothrips signipennis, Verticillium lecanii.*

INTRODUCTION

Maharashtra occupies prominent place in the production of banana with the production of 4.96 million metric tones from an area of 0.08 million hectare with 62.9 mt/ha productivity (Patil and Rawale, 2009). Among the different constraints in the production of banana, red rust thrips has become an important insect pest of banana fingers. Nymphs and adults feeds on the fruit, which develop into red blemishes on the fingers and cracks are developed later on. This affects the cosmetic value of fruit which greatly affects the marketability. More severe damage was observed during the months of July to September. In view of the seriousness of the this pest the present investigation was therefore undertaken to study the comparative efficacy of some selected insecticides and biopesticides for the control of red rust thrips (*Cheatanophothrips signipennis*) on banana fingers.

MATERIAL AND METHODS

A field experiment was conducted at Banana Research Station, Jalgaon during 2003-04, 2004-05 and 2005-06. Nine treatments including six insecticides, two biopesticides with untreated check were evlauated in Randomized Block Design which were replicated three times (Table 1 and 2). The banana cv. Grand Naine (AAA) was planted at 1.5 x 1.5 m in July month of every year. All the recommended agronomic practices were followed. Since thrips prefer young, growing plant tissue, direct insecticide sprays to the area of bud development were reported to be effective (Hara *et al.*, 2002), the spray schedule was followed accordingly. Therefore first spraying was done at the time of flag stage or just shooting stage and subsequent spray was given seven days after first spray. The observations on total number of fruits, damaged or infested fruits per bunch and yield of the bunch were recorded at maturity stage. The economics of different treatments were also worked out.

RESULTS AND DISCUSSION

The data of three years *viz.* 2003-04, 2004-05 and 2006-07 and pooled means were depicted in Table No.1 The data revealed that all the insecticides and biopesticides were significantly superior over untreated control. The spray treatment with acetamiprid 0.0025% was found most effective for the control of red rust thrips damage which recorded least banana fruit infestation (18.67%). This treatment was on par with monocrotophos 0.05% (20.32%), deltamethrin 0.001% (20.58%), acephate 0.1125% (21.00%), *Verticillium lecanii* @

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Table 1 : Field efficacy of various insecticides and pesticides against red rust thrips on banana fruits.

Treatment	Total no. of fruits/bunch				Infested fruits/bunch				% infestation of fruits/bunch			
Monocrotophos 0.05%	03 - 04	04 - 05	06 - 07	Mean	03 - 04	04 - 05	06 - 07	Mean	03 - 04	04 - 05	06 - 07	Mean
Dimethoate 0.03%	146.50	143.58	145.50	145.19	32.33	27.00	29.50	29.61	21.76 (27.90)	18.99 (25.82)	20.22 (26.16)	20.32 (26.59)
Acephate 0.1125%	148.17	140.75	147.67	145.53	33.67	28.42	30.42	30.84	22.97 (28.59)	20.21 (26.73)	20.60 (26.87)	21.26 (27.40)
Endosulfan 0.05%	147.42	139.42	144.33	143.72	31.42	27.67	29.92	29.67	22.38 (28.24)	19.90 (26.49)	20.73 (26.94)	21.00 (27.22)
Acetamiprid 0.125%	147.42	139.50	144.42	143.78	33.58	32.08	32.92	32.86	24.06 (28.82)	22.95 ()28.63	22.96 (28.37)	23.32 (28.61)
Deltamethrin 0.001%	145.50	140.17	145.58	143.75	30.33	24.33	25.42	26.86	21.20 (27.44)	17.35 (24.63)	17.45 (24.73)	18.67 (25.60)
NSKE 5%	148.08	139.58	143.50	143.72	32.25	27.50	28.50	29.42	21.78 (27.81)	19.70 (26.35)	20.27 (26.70)	20.58 (26.95)
Verticillium lecanii	145.75	141.33	142.08	143.05	34.33	33.08	32.33	33.25	22.57 (29.04)	23.40 (28.93)	22.73 (28.40)	22.90 (28.79)
Untreated Control	147.25	140.33	143.67	143.75	32.66	28.17	29.25	30.14	22.14 (28.17)	20.07 (26.63)	20.85 (27.07)	21.11 (27.29)
S.E.±	147.00	141.42	143.42	143.95	123.83	111.58	108.75	114.78	86.18 (68.16)	78.33 (62.25)	75.81 (60.47)	80.11 (63.63)
C.D. at 5%	0.82	1.36	1.40	0.75	0.36	0.84	0.91	1.34	0.41	0.40	0.51	0.95 (0.68)
	NS	NS	NS	NS	1.09	2.51	2.76	4.02	1.24	1.21	1.53	2.84 (2.04)

* Figures in parenthesis shows arcsine values.

Table 2 : Effect of different treatments on monetary returns.

Treatment	Total Yield (mt/ha)	Good Quality yield (%)	Low Quality yield (%)	Good Quality yield (mt/ha)	Low Quality yield (mt/ha)	Monetory Returns of Good Quality yield (Rs)	Monetory Returns of Low Quality yield (Rs)	Total Monetory Returns (Rs./ha)	Cost of Cultivation + treatment cost (Rs./ha)	CBR
Monocrotophos 0.05%	67.95	79.68	20.32	54.52	13.92	190938.30	24290.00	215228.30	151475	1:1.42
Dimethoate 0.03%	68.28	78.74	21.26	53.64	14.50	187791.70	25214.34	213006.04	151178	1:1.41
Acephate 0.1125%	68.45	79.00	21.00	53.59	14.26	187848.30	24873.00	212721.00	151925	1:1.40
Endosulfan 0.05%	68.90	76.68	23.32	51.83	15.77	181616.70	27543.34	209160.04	151438	1:1.38
Acetamiprid 0.0025%	68.39	81.33	18.67	56.63	13.00	198655.00	22536.67	221192.00	151750	1:1.46
Deltamethrin 0.001%	68.21	79.42	20.58	54.16	14.05	189633.30	24493.34	214126.64	151038	1:1.42
NSKE 5%	68.08	77.10	22.90	52.40	15.56	183228.30	27073.34	210301.64	150975	1:1.39
Verticillium lecanii	68.08	78.89	21.11	53.80	14.40	188421.70	25104.34	213526.04	152085	1:1.40
Untreated Control	45.75	19.89	80.11	13.47	54.19	48335.00	94289.34	142624.34	150000	1:0.95
S.E.±	7.54	0.95	0.95	0.74	0.62	2373.03	1242.38	- -	- -	0.01
C.D. at 5%	NS	2.84	2.34	2.13	1.85	7108.19	3723.00	- -	- -	0.03

Selling rate of Banana: Rs. 3500/mt for good quality
Rs. 1750/mt for low quality

$2x10^8$ cfu/ml) 3 g/lit (21.11%) and dimethoate 0.03% (21.26%). The treatment with endosulfan 0.03% (23.32%) and NSKE 5% (22.90%) was found least effective in controlling damage of red rust thrips. Padamanabhan and Mustaffa (2008) reported that the use of 0.001% Imidacloprid (1.8 EC) was effective for the control of red rust thrips. Hara *et al.*, (2002) was of the opinion that the direct insecticidal sprays are effective in controlling red rust thrips. Thus the results obtained with acetamiprid (0.0025%) are in general agreement with Padmanabhan and Mustaffa (2008). Amongst the biopesticides *Verticillium lecanii* (@ 2 x 10^8 cfu/ml) 3 g/lit was more effective than NSKE 5% in reducing damage of red rust thrips. Padmanabhan (2009) had also recommended the use of *Verticillium lecanii* and Azadirachtin 1%.

As regards the yield, there were no significant difference among the treatments, but in respect of monetary returns due to treatments (Table No. 2) acetamiprid (0.0025%) recorded highest monetary returns of Rs. 221192.70 which also had highest cost benefit ratio of 1.42.

From an expletory trial to find out the comparative effectiveness of different insecticidal treatments, acetamiprid (0.0025%) was found to be most effective treatment for the control of red rust thrips damage. However biopesticides namely NSKE 5% or *Verticillium lecanii* 3 g/liter has also recorded the desired level of reduction in infestation of red rust thrips (21.11% and 22.90%). Therefore both these biopesticides as an ecosafe measure can be included in the

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pest management programme for reduction in the attack of red rust thrips on banana fingers.

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Studies on the Effect of Foliar Application of Organic Nutrients on the Growth, Flowering and Flower Quality of Dendrobium Orchid cv. Sakura Pink

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An experiment was conducted in order to study the effect of foliar application of organic nutrients on the growth, flowering and flower quality of Dendrobium orchid cv. Sakura pink in CRD comprising eighteen treatments with four replications during 2004-2006 in the Department of Horticulture, Annamalai University, Annamalainagar, Tamil Nadu, India. Charcoal, brick pieces and coconut husk have been used as potting media for nourishing the plants. Foliar organic nutrients *viz*, vermiwash (1% and 2%), panchakavya (1% and 2%), humic acid (0.2% and 0.3%) and tender coconut water (1 : 1 and 1 : 2 dilution) were sprayed at four days and eight days interval. A treatment comprising recommended dose of chemical fertilizers was used as check and no fertilizer application as control. The results revealed that vermiwash 1% at 4 days interval found to be optimum for flower quality of Dendrobium orchid cv. Sakura pink. Even though recommended dose of chemical fertilizers recorded higher values in vegetative growth and flowering characters, it was on par with the plants treated with vermiwash 1% at 4 days interval.

Keywords : Dendrobium, Foliar application, Orchids, Organic nutrition, Flower qualtiy.

INTRODUCTION

Orchid flowers are known for the exquiste beauty, brilliance of colours, remarkable range of sizes, manifold shapes, variation in the form, attractive habits and wide distribution in the earth which have collectively aroused the highest admiration these charming plants through out the world (Battacharjee and Mukherjee 1981 and Upadhyaya *et al.*, 2006). At present tropical orchid production gains momentum in India. Among different orchids, Dendrobium accounts for abouts 80 per cent of the total tropical orchids. Orchid cut flower industry has become a well developed and specialized trade. Growth and flowering of orchids are improved by a regular schedule of fertilizing the plants in liquid form (Bose and Battacharjee, 1972). Hence, water soluble chemical fertilizers are commonly used for orchid cultivation. But, reports conform that the produces from the organic farming are good in quality and liquid extract of some of the organic nutrients like vermiwash, panchakavya and humic acid are to promote the quality of many flower crops [Sabina George and Mohanakumaran, 1999 and Pant and Singh, 2004]. Hence, an attempt was made to study the effect of organic foliar nutrition and its frequency on growth, flowering and flower quality of Dendrobium orchid cv. Sakura Pink.

MATERIAL AND METHODS

The present investigation was conducted during 2004-2006 in the Department of Horticulture, Annamalai University, Annamalainagar, Tamil Nadu, India. The experiment site is located at 6 Km west of Bay of Bengal, 11°24' North latitude, 74°11' East longitude and at an altitude of 5.59 m above MSL with the mean maximum temperature of 33.14°C, mean minimum temperature of 24.6°C, average relative humidity of 88 per cent and annual rainfall of 1897.4 mm.

The experiment was laid out in CRD comprising 18 treatments with four replications. Charcoal, brick pieces and coconut husk have been used as a potting media for nourishing the plants. Foliar organic nutrients *viz.*, vermiwash (1 and 2%), panchakavya (1% and 2%), humic acid (0.2% and 0.3%) and tender coconut water (1 : 1 and 1 : 2 dilution) were sprayed at four and eight days interval. A treatment comprising recommended dose of chemical fertilizers was used as check and no fertilizer application as control. The vegetative parameters like plant height, number of leaves per plant, leaf area, number of shoots per plant, dry matter production and quality characters like number of days for flower bud appearance, number of days for flower bud opening from flower bud appearance, length of flower stalk, number of florets per stalk, number of florets stalks per plant, florets diameter,

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Table 1 : Effect of organic foliar nutrition on growth attributes of Dendrobium orchid cv. Sakura Pink.

Treatments	Plant height (cm)	No. of leaves per plant	Leaf area (cm^2)	Dry matter production (g)	No. of shoots per plant	No. of days for f.b.a	No. of days for .f.b.o. from f.b.a	No. of flower stalks per plant
T_1 - Vermiwash 1% at 4 days interval	42.76	8.30	45.23	9.66	4.72	186.30	20.66	3.00
T_2 - Vermiwash 1% at 8 days interval	34.08	6.27	34.31	7.85	3.57	225.90	24.86	2.08
T_3 - Vermiwash 2% at 4 days interval	40.25	7.79	41.62	8.94	4.27	195.33	21.77	2.27
T_4 - Vermiwash 2% at 8 days interval	36.72	6.92	37.93	8.16	3.69	208.53	23.12	2.48
T_5 - Panchakavya 1% at 4 days interval	32.48	5.85	31.91	7.33	3.45	221.73	24.52	2.15
T_6 - Panchakavya 1% at 8 days interval	41.87	8.09	44.03	9.42	4.47	189.66	21.02	2.92
T_7 - Panchakavya 2% at 4 days interval	34.95	6.50	35.53	7.62	3.50	214.34	23.88	2.31
T_8 - Panchakavya 2% at 8 days interval	30.70	5.36	29.48	6.94	3.22	232.98	25.55	1.93
T_9 - Humic acid 0.2% at 4 days interval	37.59	7.11	39.14	8.41	3.94	204.75	22.76	2.52
T_{10} - Humic acid 0.2% at 8 days interval	38.48	7.36	40.35	8.54	4.02	201.99	22.48	2.62
T_{11} - Humic acid 0.3% at 4 days interval	29.82	5.17	28.27	6.83	3.14	239.25	25.91	1.85
T_{12} - Humic acid 0.3% at 8 days interval	35.84	6.71	36.75	8.01	3.62	211.63	23.54	2.40
T_{13} - Tender coconut water (1 : 1 dilution) at 4 days interval	41.13	7.89	42.84	9.20	4.39	192.55	21.36	2.83
T_{14} - Tender coconut water (1 : 1 dilution) at 8 days interval	39.35	7.56	41.43	8.72	4.15	198.73	22.09	2.70
T_{15} - Tender coconut water (1 : 2 dilution) at 4 days interval	31.57	5.63	30.71	7.11	3.34	230.04	25.20	2.00
T_{16} - Tender coconut water (1 : 2 dilution) at 8 days interval	33.19	6.04	33.10	7.49	3.48	217.75	24.13	2.23
T_{17} - Chemical nutrition	43.19	8.39	46.16	9.82	4.80	184.32	20.90	3.03
T_{18} - Control	28.81	4.82	26.90	6.49	2.88	243.23	26.42	1.73
SEd	0.42	0.08	0.59	0.1	0.24	1.33	0.16	0.02
CD (p=0.05)	0.86	0.18	1.18	0.21	0.48	2.68	0.32	0.05

f.b.a. – Flower bud appearance

f.b.o. – Flower bud opening

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Table 2 : Effect of organic foliar nutrition on flowering and flower quality characters of Dendrobium Orchid cv. Sakura Pink.

Treatments	Length of flower stalk	No. of florets per stalk	Floret diameter (cm)	Longevity of flower stalks in the plant (days)	Vase life of flower stalks (days)
T_1 - Vermiwash 1% at 4 days interval	29.42	8.36	7.56	48.89	22.96
T_2 - Vermiwash 1% at 8 days interval	24.90	6.23	6.80	36.77	18.69
T_3 - Vermiwash 2% at 4 days interval	27.66	7.62	7.05	42.06	21.71
T_4 - Vermiwash 2% at 8 days interval	24.81	7.00	6.55	39.74	20.42
T_5 - Panchakavya 1% at 4 days interval	22.51	6.40	5.93	37.41	18.94
T_6 - Panchakavya 1% at 8 days interval	28.86	8.20	7.42	48.17	22.56
T_7 - Panchakavya 2% at 4 days interval	23.64	6.73	6.26	38.79	19.78
T_8 - Panchakavya 2% at 8 days interval	20.76	5.90	5.40	35.49	17.85
T_9 - Humic acid 0.2% at 4 days interval	25.36	7.16	6.62	40.39	19.52
T_{10} - Humic acid 0.2% at 8 days interval	25.96	7.35	6.76	41.06	21.03
T_{11} - Humic acid 0.3% at 4 days interval	20.15	5.72	6.26	34.82	17.46
T_{12} - Humic acid 0.3% at 8 days interval	24.23	6.91	6.41	39.01	20.20
T_{13} - Tender coconut water (1 : 1 dilution) at 4 days interval	27.61	7.83	7.20	42.11	21.95
T_{14} - Tender coconut water (1 : 1 dilution) at 8 days interval	26.49	7.51	6.90	41.40	21.48
T_{15} - Tender coconut water (1 : 2 dilution) at 4 days interval	21.34	6.05	5.66	36.12	18.26
T_{16} - Tender coconut water (1 : 2 dilution) at 8 days interval	23.09	6.56	6.07	38.07	19.35
T_{17} - Chemical nutrition	25.18	7.01	7.08	38.07	20.16
T_{18} - Control	19.43	5.43	5.06	33.88	16.95
SEd	0.26	0.06	0.05	0.30	0.18
CD (p=0.05)	0.54	0.14	0.12	0.62	0.38

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longevity of flower stalk in the plant and vase life of flower stalk were recorded. The data were statistically analyzed by adopting the procedure of Panse and Sukhatme (1978).

RESULTS AND DISCUSSION

The results on the effect of organic foliar nutrients on the growth characters of orchid in comparison with inorganic nutrients are presented in Table 1. All the growth characters are significantly and positively influenced by the organic and inorganic nutrients in comparison to the control. However, the maximum plant height, number of leaves per plant, leaf area, number of shoots per plant and dry matter production were noticed in a chemical fertilizer treated plants (T_{17}) which were on par with 1% vermiwash spray at 4 days interval (T_1). The controlled plants (T_{18}) exhibited the minimum plant growth. The higher dose of vermiwash and other organic nutrients in different frequency of application also improved the growth of Dendrobium orchids over control. These results revealed that vermiwash is capable of replacing the inorganic nutrients in orchid growth and development as reported by Pant and Singh (2004). Choudhary *et al.* (2004) reported that vermiwash contains major and minor nutrients, enzymes, antibiotics, vitamins, beneficial micro organisms and other plant growth hormones and have definite advantage over other organic nutrients in respect of plant growth and development. This result is in confirmatory with the above findings.

The flower yield characters such as number of flower stalks per plant, number of days for flower bud appearance, and number of days for flower bud opening from flower bud appearance were significantly influenced by the treatments (Table 1). Among the different treatments, chemical fertilizer treatment (T_{17}) exhibited the higher value which was on par with vermiwash 1% spray at four days interval (T_1) for all the characters, while the other treatments exhibited significantly lower values.

With regarding the quality characters such as stalk length, number florets per stalk, floret diameter, longevity of flower stalk in plant and vase life (Table 2), the vermiwash 1% spray at four days interval was found to be the superior compared to chemical fertilizers. The control treatment exhibited the minimum value. The other organic nutrients such as panchakavya, humic acid and tender coconut water were also performed well over chemical treatments. This might be due to the positive influence of organic nutrients on the quality of produce as reported by Pant and Singh (2004).

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Influence of Soil and Foliar Application of Organic Nutrients on Yield Performance of Cucumber

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An investigation was carried out to find out effect of soil and foliar application of organic nutrients on the yield performance of cucumber. Among the various organic nutrients, application of FYM 12.5 t ha^{-1} + vermicompost 2.5 t ha^{-1} and 3 per cent panchakavya as foliar spray was considered as the best treatment, followed by recommended dose of fertilizers (80 : 50 : 50 Kg NPK ha^{-1}). Among the treatment combination, application of FYM 12.5 t ha^{-1} + vermicompost 2.5 t ha^{-1} and 3 per cent panchakavya as foliar spray to cucumber *cv.* Long Green favourably increased the yield components *viz.*, number of fruits per vine (14.56 in tender fruit, 10.89 in matured fruit), single fruit weight (50.72 g in tender fruit; 752.07 g in matured fruit), fruit yield per vine (71.70 g in tender fruit; 6792.07 g in matured fruit). The lowest value was recorded in absolute control.

Keywords : FYM, Vermicompost, Yield, Cucumber.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most important crop of the family cucurbitaceae. Its cultivation in India has been practiced since long. The area under cucumber in India is estimated to be 212.0 hectares with a total of production about 2544.0 tonnes. (Brij Bala and Sharma, 2006). It is one of the quickest maturing vine vegetable crop. The tender fruits are eaten raw with salt as salad and also used in culinary preparations. The fruits are rich in vitamin B, vitamin C and minerals such as phosphorus, potassium, calcium and iron (Bose and Som, 1986).

Organically grown horticultural produces are residue free and fetches a high price in the market. In recent years, organic farming is becoming more popular in India because people are now aware about the disastrous side effects caused by chemical farming on health and are now performing organically grown foods (Rajagopal and Sreeramulu, 1999). Not much study on the above line has been attempted in cucumber to suit the coastal eco-system like ours. With this background in view, the present study has been taken up to find out the effect of organic nutrients on yield and yield attributes of cucumber.

MATERIAL AND METHODS

The present investigation was carried out in the organic plot maintained at Vegetable field unit of Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalainagar during two seasons *viz.*, Season-I (July-September, 2006) and Season-II (January-March, 2007) under irrigated conditions.

The experiment was laid out in a Randomized Block Design with 14 treatments and 3 replications with a spacing of 1 x 1 m. An early variety long green with long fruits of 30 - 40 cm length, light green skinned, fleshy and crisp has been selected for the study.

The treatment comprised of T_1 - Absolute control, T_2 - FYM @ 25 t ha^{-1}, T_3 - FYM @ 25 t ha^{-1} + panchakavya 3%, T_4 - Seaweed extract @ 25 Kg ha^{-1}, T_5 - Seaweed extract @ 25 Kg ha^{-1}+ panchakavya 3%, T_6 - Vermicompost @ 5 t ha^{-1}, T_7 - Vermicompost @ 5 t ha^{-1} + panchakavya 3%, T_8 - FYM @ 12.5 t ha^{-1} + Seaweed extract @ 25 Kg ha^{-1}, T_9 - FYM @ 12.5 t ha^{-1} + Seaweed extract @ 25 Kg ha^{-1}+ panchakavya 3%, T_{10} - FYM @ 12.5 t ha^{-1} + Vermicompost @ 2.5 Kg ha^{-1}, T_{11} - FYM @ 12.5 t ha^{-1} + Vermicompost @ 2.5 Kg ha^{-1} + panchakavya 3%, T_{12} - Seaweed extract @ 25 Kg ha^{-1} + Vermicompost @ 2.5 Kg ha^{-1}, T_{13} - Seaweed extract @ 25 Kg ha^{-1} + Vermicompost @ 2.5 Kg ha^{-1} + panchakavya 3%, T_{14} - Recommended dose of inorganic fertilizers alone (80 : 50 : 50 Kg NPK ha^{-1}).

Each treatment was replicated thrice and four plants were randomly selected and maintained each for tender and matured fruits. Observations were recorded on number of fruits per vine, single fruit weight and fruit yield. The number of fruits produced per vine, harvested both at tender and matured stage throughout the total cropping period were counted and recorded. Harvested tender and matured marketable fruit were weighed individually and the mean was worked out and

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Table 1 : Effect of soil and foliar application of organic nutrients on number of fruits per vine in cucumber cv. Long Green.

Treatments	Season I		Season II	
	Tender fruit	Matured fruit	Tender fruit	Matured fruit
T_1 - Absolute control	7.52	6.22	6.80	6.15
T_2 - FYM @ 25 t ha^{-1}	10.21	8.02	9.10	8.02
T_3 - FYM @ 25 t ha^{-1} + panchakavya (PK) 3%	12.86	9.79	12.30	9.62
T_4 - Seaweed extract @ 25 Kg ha^{-1}	7.55	7.06	7.09	6.42
T_5 - Seaweed extract @ 25 Kg ha^{-1} + PK 3%	8.84	7.55	8.69	6.98
T_6 - Vermicompost (VC) @ 5 t ha^{-1}	9.24	8.78	8.94	7.21
T_7 - VC @ 5 t ha^{-1} + PK 3 %	12.13	8.01	11.00	7.09
T_8 - FYM @ 12.5 t ha^{-1} Seaweed extract @ 25 Kg ha^{-1}	9.18	8.53	8.54	7.09
T_9 - FYM @ 12.5 t ha^{-1} Seaweed extract @ 25 Kg ha^{-1} + PK 3%	8.98	8.36	9.86	7.52
T_{10} - FYM @ 12.5 t ha^{-1} + VC @ 2.5 t ha^{-1}	12.42	9.61	12.52	9.42
T_{11} - FYM @ 12.5 t ha^{-1} + VC @ 2.5 t ha^{-1} + PK 3 %	14.56	10.89	14.25	10.76
T_{12} - Seaweed extract @ 25 Kg ha^{-1} + VC @ 2.5 t ha^{-1}	9.32	8.91	9.09	7.00
T_{13} - Seaweed extract @ 25 Kg ha^{-1} + VC @ 2.5 t ha^{-1} + PK 3%	10.56	8.58	10.22	8.24
T_{14} - RDF (80 : 50 : 50 Kg NPK ha^{-1})	13.69	10.33	13.10	10.25
SED	0.40	0.26	0.39	0.25
CD (p=0.05)	0.81	0.53	0.78	0.50

RDF-Recommended dose of inorganic fertilizers alone.

Table 2 : Effect of soil and foliar application of organic nutrients on single fruit weight (g) in cucumber cv. Long Green.

Treatments	Season I		Season II	
	Tender fruit	Matured fruit	Tender fruit	Matured fruit
T_1 - Absolute control	45.42	510.23	44.38	508.21
T_2 - FYM @ 25 t ha^{-1}	48.10	497.00	47.20	495.00
T_3 - FYM @ 25 t ha^{-1} + panchakavya (PK) 3%	50.21	674.56	49.23	671.57
T_4 - Seaweed extract @ 25 Kg ha^{-1}	46.43	491.25	45.24	490.29
T_5 - Seaweed extract @ 25 Kg ha^{-1} + PK 3%	46.60	492.00	45.52	491.22
T_6 - Vermicompost (VC) @ 5 t ha^{-1}	47.00	494.01	46.01	492.20
T_7 - VC @ 5 t ha^{-1} + PK 3 %	49.63	499.03	48.52	496.16
T_8 - FYM @ 12.5 t ha^{-1} Seaweed extract @ 25 Kg ha^{-1}	47.23	495.58	47.21	490.65
T_9 - FYM @ 12.5 t ha^{-1} Seaweed extract @ 25 Kg ha^{-1} + PK 3%	47.89	496.42	46.83	492.42
T_{10} - FYM @ 12.5 t ha^{-1} + VC @ 2.5 t ha^{-1}	50.00	660.26	49.00	658.13
T_{11} - FYM @ 12.5 t ha^{-1} + VC @ 2.5 t ha^{-1} + PK 3 %	50.72	752.07	49.62	748.29
T_{12} - Seaweed extract @ 25 Kg ha^{-1} + VC @ 2.5 t ha^{-1}	46.89	493.02	45.81	489.40
T_{13} - Seaweed extract @ 25 Kg ha^{-1} + VC @ 2.5 t ha^{-1} + PK 3 %	49.01	499.96	48.30	492.42
T_{14} - RDF (80 : 50 : 50 Kg NPK ha^{-1})	50.40	740.02	49.52	738.42
SED	0.09	5.84	0.09	5.84
CD (p=0.05)	0.19	11.75	0.18	11.74

RDF-Recommended dose of inorganic fertilizers alone

expressed in gram. Fruit yield per vine was calculated by adding the weight of edible fruits that were harvested from the plant during the fruiting period and was expressed in gram. The data were statistically analyzed as per the procedure given by Panse and Sukhatme (1978) wherein the results were found significant, the critical difference were worked out at 5 per cent level and statistical conclusions were drawn.

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Table 3 : Effect of soil and foliar application of organic nutrients on fruit yield per vine (g) in cucumber cv. Long Green.

Treatments	Season I		Season II	
	Tender fruit	Matured fruit	Tender fruit	Matured fruit
T_1 - Absolute control	453.60	3420.12	430.34	3300.20
T_2 - FYM @ 25 t ha^{-1}	628.46	5130.10	618.10	5116.08
T_3 - FYM @ 25 t ha^{-1} + panchakavya (PK) 3%	694.23	5462.43	672.53	5432.22
T_4 - Seaweed extract @ 25 Kg ha^{-1}	469.94	3860.08	452.32	3730.83
T_5 - Seaweed extract @ 25 Kg ha^{-1} + PK 3%	491.32	4030.10	480.45	3984.30
T_6 - Vermicompost (VC) @ 5 t ha^{-1}	553.72	4370.56	531.42	4320.10
T_7 - VC @ 5 t ha^{-1} + PK 3 %	664.23	5192.42	565.53	4610.08
T_8 - FYM @ 12.5 t ha^{-1} Seaweed extract @ 25 Kg ha^{-1}	582.00	4692.42	565.53	4610.82
T_9 - FYM @ 12.5 t ha^{-1} Seaweed extract @ 25 Kg ha^{-1} + PK 3%	600.93	5112.90	599.51	5100.18
T_{10} - FYM @ 12.5 t ha^{-1} + VC @ 2.5 t ha^{-1}	682.31	5247.50	632.43	5200.50
T_{11} - FYM @ 12.5 t ha^{-1} + VC @ 2.5 t ha^{-1} + PK 3 %	710.70	6792.10	700.10	6765.04
T_{12} - Seaweed extract @ 25 Kg ha^{-1} + VC @ 2.5 t ha^{-1}	525.38	4152.53	506.65	4110.12
T_{13} - Seaweed extract @ 25 Kg ha^{-1} + VC @ 2.5 t ha^{-1} + PK 3 %	646.00	5157.09	622.23	3157.09
T_{14} - RDF (80 : 50 : 50 Kg NPK ha^{-1})	702.62	6777.09	692.83	6752.43
SED	17.62	46.63	18.07	48.76
CD (p=0.05)	35.24	93.27	36.15	95.52

RDF-Recommended dose of inorganic fertilizers alone.

RESULTS AND DISCUSSION

The data presented in Table 1 showed the effect of organic nutrients on the fruit production in cucumber. Statistically significant differences were observed among the treatments in both the seasons. In season I among the various treatment, T_{11} was found to be the best treatment in producing the maximum number of tender and matured fruits per vine (14.56 and 10.89) followed by T_{14} (13.69 and 10.33) and T_3 (12.86 and 9.79). The control (T_1) ranked the least in the production of both tender and matured furits (7.52 and 6.22). In season II also, a similar trend was observed with treatment, T_{11} producing the maximum number of tender and matured fruits (14.25 and 10.76) followed by T_{14} (13.10 and 10.25). T_1 (control) registered the minimum number of tender and matured fruits (6.80 and 6.15 respectively).

The single fruit weight was found to vary significantly among the various treatments in both the seasons (Table 2). In season I, T_{11} recorded the maximum weight of both tender and matured fruits (50.72 and 752.07 g) followed by T_{14} (50.40 and 740.02 g) and T_3 (50.21 and 674.56g), while the minimum weight of tender fruit (45.42 gm) was observed in T_1 (control) and matured fruit (491.25 gm in T_4). In season II, among the various treatments. T_{11} registered the maximum weight of both tender and matured fruits (49.62 and 748.29 g) followed by T_{14} (49.52 and 738.42) and T_3 (49.23 and 671.57g). T_1 (Absolute control) recorded minimum fruit weight of tender fruit (44.38 g) and matured fruit (409.40g in T_{12}).

From the data presented in Table 3 it can be observed that the fruit yield was found to vary significantly among the various treatments in both the seasons. In season, I the maximum yield of both tender and matured fruits (710.70 and 6792.07 g) was registered in T_{11}, followed by T_{14} (702.53 and 6777.09 g) and T_3 (694.23 and 5462.43 g). The minimum yield of tender and matured fruits (453.60 and 3420.12 g) was observed in the control (T_1). In season II also, a similar trend was observed, with T_{11} recording the highest yield of both tender and matured fruits (700.10 and 6765.04 g), while the treatment T_{14} (692.83 and 6752.43 g) T_3 (672.53 and 5432.22 g) proved to be the next best treatments.

Amirthalingam and Balakrishnan (1988) opined that the increase in number of fruits and single fruit weight may be attributed to the increase in the number of cells as well as elongation of individual cells, which might be rendered possible through better translocation of soluble ions under optimum levels of nutrients. It was observed by Naidu *et al.*, (1999) that taller plants with more number of branches increase photosynthetic area and favourable physiological activities under higher nutrient levels could have resulted in more production and translocation of photosynthates in plants, which accelerated the formation of more number of large sized fruits resulting in higher yields.

Higher yields due to application of vermicompost may be attributed to the high level of nutrients along with growth stimulating substances excreted by earthworms into their casts. Tomati *et al.*, (1988) emphasized the influence of microbial, hormone like substances on the plant metabolism, growth and development by vermicompost.

These EMO in panchakavya would have enhanced the productivity of phytohormones like auxins and gibberellins that

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might have in turn, stimulated the growth by increasing the growth parameteres as evidenced from the work of Sivakumar (2004). In the present study also, foliar spray of panchakavya (3%) along with other organic manures like FYM (12.5 t ha^{-1}) and vermicompost (2.5 t ha^{-1}) resulted in enhancing the growth parameters.

The favourable effect of panchakavya on fruit yield may be due to the fact that panchakavya acts as a growth-promoter and immunity booster. Panchakavya stock solution creates a depression, which facilitates a cosmic ray link. The basic elements for the growth are harmonized by this energy which refresh the growth process (Sundarraman *et al.*, 2001). The results of the present study are in accordance with those of Sridhar (2003) and Sivakumar (2004) in black nightshade.

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Studies on the Effect of Planting Density and Sucker Arrangement on Growth and Flowering in Banana

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The field experiment was conducted at Banana Research Station, Jalgaon (Mahatma Phule Krishi Vidyapeeth) during 2006-2009 to study the growth performance of Grand Naine (AAA) and Basrai (AAA) under different planting densities with sucker arrangements and also to find out the suitable planting system. Results revealed that pseudo stem height tended to increase as plant population increased and tallest plants were oberved in P_1 (2 x 3 m with three suckers per hill with a plant population of 5001 plants/ha) and P_2 (1.8 x 3.6 m with three suckers per hill with a plant population of 4629 plants/ha). Pseudo stem girth and number of functional leaves decreased with increase in plant population. Highest plant population (P_1 2 x 3 m with three suckers per hill with a plant population of 5001 plants/ha) recorded maximum plant height (170.38 cm), whereas maximum plant girth (66.38 cm) and maximum number of functional leaves at flowering was observed in P_3 (pair row planting at 0.9 x 1.2 x 2.1 m with a plant population of 4444 plants/ha). Days to flowering and days to harvesting were extended with the increase in plant population. Days to flowering and days to harvesting was extended by 4.56 days and 7.25 days in P_1 (2 x 3 m with three suckers per hill with a plant population of 5001 plants/ha) planting system. Pair row planting having 0.9 x 1.2 x 2.1 m with a plant population of 4444 plants/ha appeared to be optimum plant spacing for both Grand Naine (AAA) and Basrai (AAA) cultivars of banana.

Keywords : Planting density, Sucker arrangement, Growth, Duration parameters and functional leaves.

INTRODUCTION

An ideal planting density is determined by a complex integration of cultivar to be grown, soil fertility, management level, economic considerations and growth due to weather conditions (Reddy and Patil, 2007; and Shanmugvelu *et al.*, 1992). The planting density/spacing has significant effect on growth, yield fruit quality, pest and disease incidence (Reddy and Kumar, 2009). High density planting by adopting closer spacing and by planting more number of suckers per pit has been attempted with varying success in banana. Therefore the present investigation was undertaken to assess the growth performance of Grand Naine (AAA) and Basrai (AAA) cultivars of banana under different planting densities with sucker arrangements in the drier regions of Jalgaon (Maharashtra).

MATERIAL AND METHODS

The present investigation was undertaken to assess the growth performance of two commercial cultivars of banana namely Grand Naine (AAA) and Basrai (AAA) under different planting densities with sucker arrangements for three crop cycles at Banana Research Station, Jalgaon (Mahatma Phule Krishi Vidyapeeth) during 2006-07, 2007-08 and 2008-09. The experiment was laid out int FRBD which comprised of three planting systems *viz.*, planting of 3 suckers per hill spaced at 2 x 3 m (P_1) with 5001 plants per hectare, planting of 3 suckers per hill spaced at 1.8 x 3.6 m (P_2) with 4629 plants per heactare, and pair row planting (P_3) with planting of only one sucker per pit spaced at 0.9 X 1.5 x 2.1 m. accommodating 4444 plants per hectare and two varieties namely Grand Naine (AAA) (V_1) and Basrai (AAA) (V_2) and were replicated three times. The planting was done in the month of July every year during the course of the investigation. Crop management practices were done as per the recommendations of Mahatma Phule Krishi Vidyapeeth, Rahuri. The data on pseudo stem height, pseudo stem girth, number of functional leaves per plant at the time of shooting, days to flowering and days to harvesting were recorded and data was subjected to statistical analysis.

RESULTS AND DISCUSSION

The results presented in Table 1 revealed significant influence of different planting densities with sucker arrangement on pseudo stem height, pseudo stem girth and number of functional leaves at the time of flowering. Highest pseudo stem height (170.38 cm) was observed under high plant population in P_1 planting system (planting of 3 suckers per hill spaced at 2 x 3 m with 5001 plants per hectare).

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Table 1 : Effect of planting densities on growth parameters in banana (Pooled over 3 years)

Treat-ment	Pseudo stem height (cm)				Pseudo stem girth (cm)				Number of functional leaves/ plant at flowering			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
V_1	174.42	170.53	169.97	171.64	66.55	65.67	67.88	66.70	10.53	10.88	11.48	10.96
V_2	166.35	165.02	166.20	165.86	65.01	64.37	64.88	64.75	9.49	9.15	9.38	9.31
Mean	170.38	167.78	168.09		65.78	65.02	66.38		9.97	10.02	10.43	
	P	V	P x V		P	V	P x V		P	V	P x V	
SE	0.36	0.29	0.50		0.15	0.12	0.21		0.08	0.06	0.11	
CD 5%	1.01	0.82	1.43		0.43	0.35	0.61		0.22	0.18	0.31	

Table 2 : Effect of planting densities on duration parameters in banana (Pooled over 3 years)

Treatment	Days to flowering				Days to harvesting			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
V_1	293.58	293.45	287.89	291.64	405.77	395.77	387.15	396.23
V_2	307.48	300.15	296.59	301.41	422.57	413.19	410.20	411.46
Mean	300.53	296.80	292.24		414.17	404.45	392.92	
	P	V	P x V		P	V	P x V	P
SE	0.73	0.60	1.03		0.95	0.77	1.34	
CD 5%	2.07	1.69	2.93		2.69	2.19	N.S.	

Between the varieties, Grand Naine (AAA) recorded the highest pseudo stem height. Interactions were also significant and significantly highest pseudo stem height was recorded by P_1V_1 (174.42 cm) treatment combination showing the influence of both planting density and veriety. The reverse trend was observed with respect to pseudo stem girth and there was decrease in pseudo stem girth with the increase in plant population. As revealed from the Table-1 significantly highest pseudo stem girth (66.38 cm) was observed in P_3 planting system (planting of single suker per pit spaced at 0.9 x 1.5 x 2.1 m). Between the varieties, maximum pseudo stem girth was recorded in Grand Naine (66.70 cm). Interactions were significant and maximum pseudo stem girth (67.88 cm) was observed in Grand Naine planted under pair row planting system (P_3 V_1) (planting of single sucker per pit spaced at 0.9 x 1.5 x 2.1 m). An increase in pseudo stem height had pseudo stem girth with the increase in planting density has been well documented by Premahalatha *et al.,* (2000): Apshara and Sathiamoorthy (2003); Baruha and Sharma (2000) and Hazarika and Mohan (2007).

With regard to the number of functional leaves at the time of flowering, there was decrease in number of functional leaves with the increase in plant density (Table 1). Maximum number of functional leaves (10.43) was recorded under P_3 planting system (planting of single sucker per pit spaced at 0.9 x 1.5 x 2.1 m) and between the varities Grand Naine (10.96) recorded the maximum number of functional leaves. Interactions were significant and maximum number of functional leaves (11.48) was registered in Grand Naine planted in pair row planting system (P_3 V_1) (planting of single sucker per pit spaced at 0.9 x 1.5 x 2.1 m). Reduction in number of functional leaves with the increase in plant density has been reported by Nalini *et al.,* (2000) in Robusta (AAA), Patel *et al.,* (2000) in Basrai (AAA), Hazarika and Mohan (2007) in Jahaji and Athani *et al.,* (2007) in Rajapuri (AAB). The increase in pseudo stem height and decrease in pseudo stem girth and number of functional leaves with the increase in plant population might be due to increased competition for soil moisture, nutrients and sunlight as a result of high plant population per unit area and less space provided for individual plant under increased plant population (Mandal, 1980; Hazarika and Mohan, 2007; Premahalatha *et al.*, 2000; and Baruha and Sharma, 2000).

Marked differences were also noticed in the days required for flowering as well as harvesting (Table 2). Both these parameters were delayed considerably with the increase in planting density. The results revealed that the flowering was delayed by 8.29 daya and 4.56 days in P_1 planting system (planting of 3 suckers per hill spaced at 2 x 3) and P_2 planting system (planting of 3 suckers per hill spaced at 1.8 x 3.6m), respectively as compared to spacing P_3 (Planting of single sucker per hill spaced at 0.9 x 1.5 x 2.1 m). P_1 planting system (planting of 3 suckers per hill spaced at 2 x 3) reqired maximum days for flowering (300.53 days), followed by P_2 planting system (planting of 3 suckers per hill spaced at 1.8 x 3.6 m) which required 296.80 days for flowering. Between cultivars delayed flowering was observed in cv. Basrai (310.41 days). Interactions were also significant and delayed flowering was observed in P_1V_2 treatment combination (Basrai planted at 2 x 3 m with 3 suckers per hill) whcih required 307.48 days for flowering. The same trend was observed with respect to days to harvesting and in P_1 (planting of 3 suckers per hill spaced at 2 x 3) and P_2 (planting of 3 suckers per hill spaced at 1.8 x 3.6 m) planting systems, harvesting was delayed by 21.25 days and 11.53

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days, respectively. Maximum days for harvesting (411.17 days) was recorded in P_1 planting system (planting of 3 suckers per hill spaced at 2 x 3) followed by P_2 planting system (planitng of 3 suckers per hill spaced at 1.8 x 3.6 m). Between varieties, Basari (AAA) required maximum days for harvesting (411.46 days). No significant difference were observed with respect to days to harvesting. However maximum days for harvesting (422.57 days) was registered in Basrai (V_2) planted under P_1 (planting of 3 suckers per hill spaced at 2 x 3) planting system. *i.e.*, P_1V_2 treatment combination. Ahmed and Mann (1970) also reported delayed flowering and harvesting in banana cv. Amritsagar (AAA) under closer spacing. Chundawat *et al.*, (1983) and Thippesha *et al.*, (2007) reported that association of poor penetration of light, poor flow of air and low temperature in closer spacing compared to wider spacing prolonged the vegetative phase and thereby took longer time for shooting. According to Thippesha *et al.*, (2007) the delayed flowering in high density planting is due to the more competition between plants for moisture, nutrients and solar radiation, whereas early flowering in wider planting systems with single sucker planting is due to the unfurling of leaves more frequently. This was evident from the maximum number of functional leaves under P_3 planting system (planting of single sucker per hill spaced at 0.9 x 1.5 x 2.1m). Wider spacing under this planting system might have promoted earlier flowering due to the exposure of a large number of leaves to sun, causing efficient photosynathesis and greater metabolic activites. The interaction effects showed that both the cultivars were under the influence of planting systems. Both the cultivars performed similarly at higher planting densities and their performance was consistent in all the three crop cycles. Under the drier climatic conditions of Jalgaon banana cultivar with dwarf stature and thicker stem is desired. Tellest plants with weak stem under high density planting Banana may lead to wind damage. Higher number of functional leaves per plant at the time of flowering leads to better bunch size and yield (Hazarika and Sharma, 2007) and maximum number of functional leaves were reported under P_3 planting system (planting of single sucker per hill spaced at 0.9 x 1.5 x 2.1 m). Delayed flowering and harvesting leads to increase in input costs like fertilizers, props, laboru etc.

The results of the present study revealed that pair row planting system with planting of single sucker per hill spaced at 0.9 x 1.5 x 2.1 m appeared to be the optimum planting system for both Grand Naine (AAA) and Basrai (AAA) cultivars of banana under ecological conditions of Jalgaon (Maharashtra).

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Ecological Status of Medicinal Plants of Durg NagarNigam Area; Chhattisgarh

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The present paper analysis native medicinal plants and their ecological status of the plant species of Nagar Nigam Area, District Durg has been abstracted synthetically. During this study one hundered fifty two species of medicinal plants belonging to sixity six families are described out of which one hundred fifty medicinal plant species belonging to sixty four different angiospermic families & two gymnosperms were found in the study area.

Keywords : Medicinal Plants, Ecological Status.

INTRODUCTION

The study of plant medicines was first described precisely in the basic literature *Charak Samhita* of Ayurvedic medicine in India. Charak, the author of *C. Samhita* flourished in the 8th century B.C. By the 16th century, a fundamental turning point reached in the history of medicinal plants with the advent of the Swiss-German physician Paracelsus (1439-1541), who gave the concept of what is now defined as 'active principles' of plants. 'Western' medicine has largely confined itself to the isolation or synthesis of single active ingredient for the treatment of specific disease (Bland, 1983). For a long time, plants have been the almost exclusive therapy available to humans. With the development of medicinal chemistry in the early 19th century, plants were also found source of substances to be developed as drugs.

In India, medicinal plants have traditionally occupied an important position in the socio-cultural and spiritual arena of rural and tribal lives. Medicinal plants are potential renewable natural resources. It has been estimated that around 8000 species, accounting for around 50 per cent flowering plant species of India, are used for production of different medicines. There are about 25000 effective plant based formulations used in folk medicine in rural communities all over India and about 10000 designed formulations are available in medicinal texts. All medicinal preparations were designed from plants either in the simple form or the most complex form of crude extracts mixtures. A large proportion of the active ingredients and chemical compounds of a particular medicinal plant, *i.e.*, drugs has been discovered with the aid of the ethno-botanical knowledge and traditional uses of the plants.

Many countries organize ethnobotanic expeditions for gathering information about traditionally used medicinal plants with the aim of using this information for developing new pharmaceuticals (de Sousa Araujo *et al.*, 2008; Gonzalez-Tejero *et al.*, 2008; Leporatti and Impieri, 2007). Recently, many developing countries have engaged into studies of traditional medicine, devoting significant attention to migrant communities in industrialized countries (Pieroni and Giusti, 2008). However, Western Europe studies have been scarce in this field (Pieroni and Gray 2008; Pieroni *et al.*, 2008). Information about medicinal plants traditionally used for therapeutic purposes is mainly deficient because archive material is not systematized and mostly presented in small ethnographic papers published in native languages (Luczaj) and Szymanski, 2007). Researchers have been discussing about integration of traditional medicine into the public health system (Alves and Rosa, 2007), which would result the need on the accumulation, systematization and presentation of material on the traditional medicine to the wider audience. Viewing these points the present investigation has planned to study the ecological status especially of the medicinal plants.

Study area : For present study the data was collected randomly from various parts of Nagar Nigam Area {District Durg} Chhattisgarh. The Durg District is a populous District of Chhattisgarh lies between 20°23' to 22°02' North Latitude & 80°46' to 81°58' East Longitude. It occupies the southwestern part of the Chhattisgarh plain and possesses belts of hilly country in the south, southwest and northwest, bestowed with mineral resources and forests. The forest area is 8.95% of Geographical Area *i.e.*, 764.46 Sq. KM. The climate of District Durg generally has a dry tropical weather which is moderate but on a warmer side in summer season. The peak temperatures are usually reached in May/June and can be as high as 46°C. The onset of monsoon is usually from July and the season extends up to September, with monsoon peaking during July and August. After monsoons, the average temperature varies between 32°C and approx 12°C right through December and January. Maximum, Average & Minimum Rain fall of District Durg in 2009 is 1487.2 mm, 1071.16 mm and 781.5 mm respectively.

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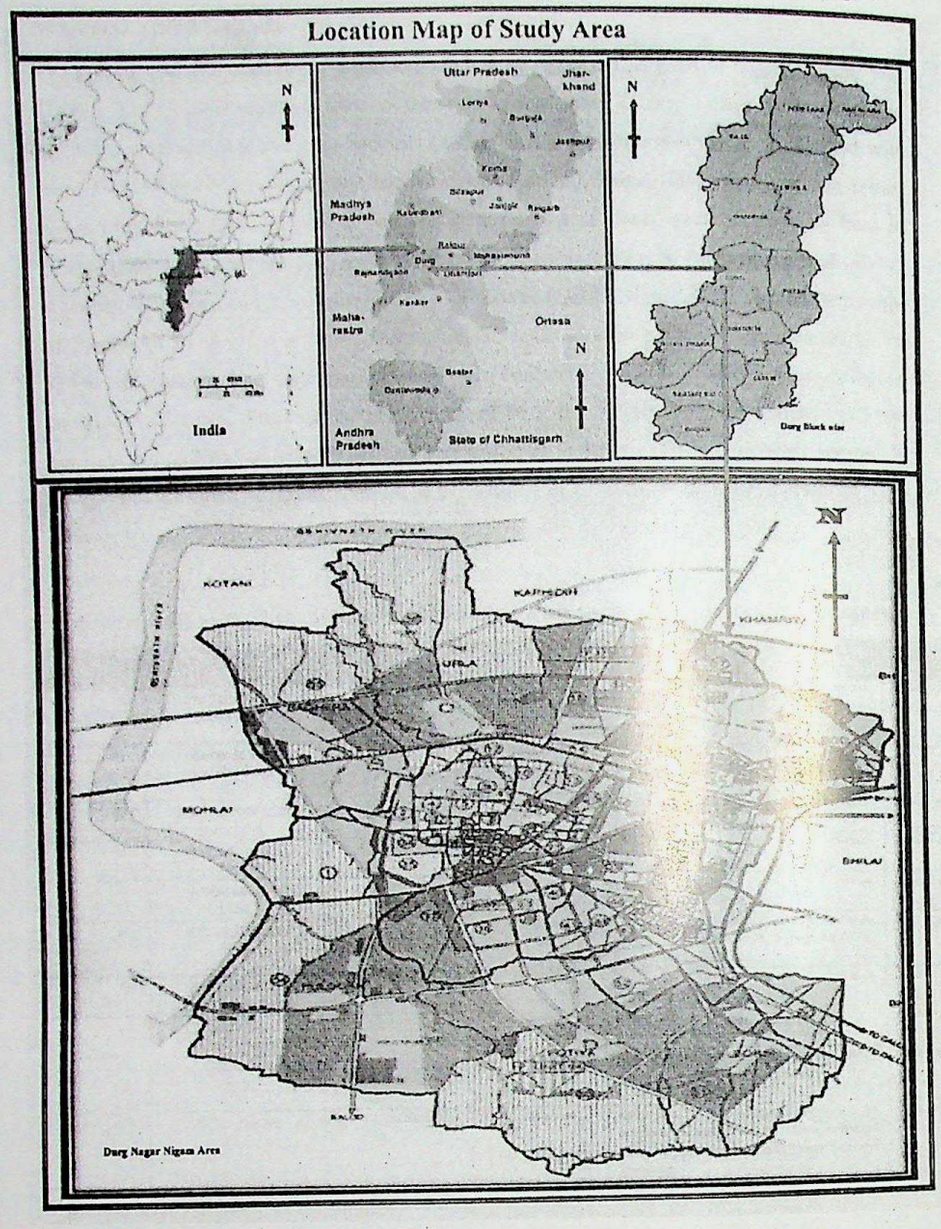

MATERIAL AND METHODS

The present paper is outcome of extensive field survey of different parts of Nagar Nigam Area {District Durg} Chhattisgarh at frequent intervals during 2008-2009 to collect information's on medicinal uses of different plant species. During field work, interviews were conducted with local knowledgeable people, herbal healers called vaidhraj, baiga, (local physicians in Indian System of Medicines), old man & women. Identification of plants was also done by flora of Haines (1961), Patwardhan & Hopper (1992), Pushpangdan (1994) & Patwardhan *et al.*, (2004). Voucher specimens of plants were collected, preserved, snaps were taken & submitted in the herbarium of Botany Department. The medicinal value of each plant was enumerated in the following patterns; The data thus obtained was computed for quantitative phytosociological characters & for the Presence is then expressed by a five degree scale as under-a) Rare {present in 1 to 20%} b) Seldom present {present in 21 to 40%} c) Oftenly present {present in 41 to 60%} d) Mostly present {present in 61 to 80%} e) Constantly present {present in 81 to 100%}.

RESULT AND DISCUSSION

During the study of Durg Nagar Nigam Area, One hundred fifty two species of medicinal plants belonging to sixty six families are described for their medicinal value as well as ecological status (Table No. - 1). One hundred fifty medicinal plant species belonging to sixty four different angiospermic families & two gymnosperms were found in the study area.

Forty eight medicinal plant species belonging to thirty two different angiospermic families & one to gymnosperm were observed in the category of rare present. Forty two medicinal plant species belonging to thirty different angiospermic families & one to gymnosperm were in the category of seldom present. Thirty two medicinal plant species belonging to twenty three different angiospermic families were in the category of oftenly present. Twenty medicinal plant species belonging to seventeen different angiospermic families were in the category of mostly present. Ten medicinal plant species belonging to nine different angiospermic families were in the category of constantly present. These plant species seem to be the

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Table 1 : List of medicinal plants of the area with their uses and ecological status.

S.No.	Botanical Name	Local Name	Family	Uses	Ecological status
1	*Abutilon indicum* (Link) Sweet	Kanghi	Malvaceae	Leaves demucent, gonorrhea & chronic brouchitis, bleeding piles; Roots: fever, leprosy, Bark: diurentic; Seed: cough.	Seldom present
2	*Acacia arabica* (Willd.) Sans	Babul	Mimosaceae	Pelia: antiseptic: Stem Bark: mouth wash, cleaning teeth; Stem: dysentery; Bark: astringent.	Constantly present
3.	*Achyranthes aspera* (Linn.) Sans	Chirchita/Latjira	Amaranthaceae	Plant: piles, colic, boils, diuretic & astringent; Roots: pyrrhoea, cough and fevers.	Mostly present
4.	*Acalypha indica* Linn.	Kuppu/Khokali	Euphorbiaceae	Flowers: diarrhea; Leaves: scabies, stomach ulcers, bed sores.	Oftenly present
5.	*Acorus calamus* Linn.	Buch/Bauj	Araceae	Rhizomes: carminative, stimulant, dyspepsia, diarrhea, fevers and bronchitis.	Seldom present
6.	*Adhatoda vasica* Ness	Arusi/Bansa	Acanthaceae	Leaves : chronic bronchitis & asthma; Flower & Fruits: antispas-modic; Roots: malaria fevers, diptheria and gororrhea.	Rare present
7.	*Aegle marmelos* (Linn.) Corr. Serr.	Bael	Rutaceae	Fruit : astringent, antiscorbutic & digestive, diarrhea and dysentery.	Seldom present
8.	*Albizia lebbeck* (Linn.) Benth.	Kokko/Siris	Fabaceae	Seeds : astringent, piles.	Seldom present
9.	*Allium cepa* Linn.	Piyaz	Alliaceae	Bulb : diuretic expectorant, piles, cough, jaundice, dyspepsia, colic, scurvy & as a sedative.	Rare present
10.	*Allium sativum* Linn.	Lahasun	Alliaceae	Bulb : diuretic & astringent, fevers, coughs, flatulence, disorders of the nervous system, pulmonary phthisis, whooping cough & dilated bronchitis.	Rare present
11.	*Aloe vera* (Linn.) Burm. f.	Gheekunvar	Liliaceae	Leaves : cough, cold, fevers, skin diseases, gonorrhea, piles, constipation, menstural suppresion jaundice, rheumatic affections, abdominal tumors & dropsy.	Oftenly present
12.	*Alstonia scholaris* R.Br.	Sapttapam/ Chhatwan	Apocyananceae	Leaves Bark: ulcers, beriberi, dropsy malaria, gastro-intestinal troubles, dysentery.	Oftenly present
13.	*Anacyclus pyrethrum* (Linn.) Link	Akarkara	Asteraceae	Plant : cardiac stimulant, rheumatism.	Rare present
14.	*Andrographis paniculata* (Burm.f.) Wall. ex Nees	Kalmegha	Acanthaceae	Plant : bronchitis, dyspepsia, dysentery, influenza; Seeds fevers.	Rare present
15.	*Annona squamosa* Linn.	Sittaphal	Annonaceae	Roots: strong purgative; Leaves poultice to ripen wounds; Seeds: insecticidal, to destory hair lice.	Mostly present
16.	*Anthocephalus cadamba* (Roxb.) Miq.	Kadamb	Rubiaceae	Bark: astringent, in snake bite; Leaves: antiseptic, diabetes.	Rare present
17.	*Argemone mexicana* Linn.	Siyal kanta	Papaveraceae	Plant juice: dropsy & jaundice; Leaf juice: scabies; Stem juice: healing of wounds, eye diseases. Seed oil: dropsy, jaundice and skin diseases.	Seldom present

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18.	*Artocarpus heteroophyllus* Lam.	Kathal	Moraceae	Leaves: sking troubles; Roots: diarrhea; Fruit juice: glandular swellings.	Rare present
19.	*Asparagus racemosus* Willd.	Satavar/ Shatavari	Asparagaceae	Roots: antiseptic, good anti diarrhea.	Oftenly present
20.	*Asplenium trichomanes* Linn.	Maidenhair spleenwort	Aspleniaceae	Plant: expectorant; Leaves: head cold and chest pains.	Rare present
21.	*Azadirachta indica* A.Juss.	Neem	Melianceae	Leaves : carminative, expectorant, anthelmintic, antidotal, diuretic; Leaves fresh juice: intestinal worms, jaundice & skin diseases; Fruit : (UTI) Piles, leprosy; Seed: leprosy and chronic diseases.	Mostly present
22.	*Bacopa monnieri* (Linn.) Pennell	Nirabrahmi	Scrophulariaceae	Dried leaf: asthenia (nervous breakdown), bronchitis and rheumatism.	Seldom present
23.	*Bambusa bambos* (Linn.) Voss	Bans/Bamboo	Poaceae	Leaf juice vomiting of blood; Shoot young: lung disease.	Seldom present
24.	*Barleria prionitis* Linn.	Vajardanti	Acanthaceae	Root: boils & swellings; Dried bark; cough.	Oftenly present
25.	*Barleria cristata* Linn.		Acanthaceae	Plant: diuretic and blood purifier.	Rare present
26.	*Basella rubra* Roxburgh	Poi bhaji	Basellaceae	Leaf: utricaria in women.	Rare present
27.	*Bauhinia purpurea* Linn.	Kolari/Koenar	Fabaceae	Flower buds: diarrhea; Roots: carminative.	Rare present
28.	*Bauhinia variegata* Linn.	Kachnar	Fabaceae	Flower powder: haemorrhage; Bark: skin diseases.	Rare present
29.	*Bidens pilosa* Linn.	Broom stick/ Chor pushpi	Asteraceae	Plant: coughs; Leaves: antiseptic, ulcers, swollen glands, eye & ear troubles.	Mostly present
30.	*Bombax ceiba* Linn.	Semal	Malvaceae	Bark: diarrhea, dysentery & menorrhagia; Fruits: snake bite; Flower buds: colitis.	Rare present
31.	*Brassica campestris* (Linn.) Wild turnip	Kalisarson	Brassicaceae	Oil: rheumatic pains, skin disease, snake bite, bodyache, Piles, Pyorrhoea, Itching, Urticaria	Seldom present
32.	*Brassica juncea* (Linn.) Czern.	Mohari/Rayi	Brassicaceae	Seed: absesses, colds, lumbago, rheumatism and stomach disorders, tumors, arthritis, foot ache, lumbago and rheumatism.	Rare present
33.	*Bryophyllum pinnatum* (Lam.) Oken.	Ghamari/ Patharchita	Crassulaceae	Leaves: boils, wounds, bites by venomous insects, potent antiseptic, kidney stones.	Rare present
34.	*Butea monosperma* (Lam.) Taub.	Palas	Fabaceae	Seed: diarrhea; Flower & Leaves: boils, pimples, colic worms & piles; Gum: diarrhea; Bark: snake bite.	Rare present
35.	*Calotropis gigantea* (Linn.) W.T. Aiton	Madar	Asclepiadaceae	Flower: cold, cough, asthma; Root bark; Dysentry; Leaves; Paralysis.	Mostly present
36.	*Calotropis procera* (Aiton) Ait. f.	Ak	Asclepiadaceae	Leaves: dropsy, skin-disease: Flower: cough, cold, asthma.	Constantly present
37.	*Carica papaya* Linn.	Papita	Caricaceae	Fruit : anthelmintic; Plant: blood anticoagulant	Mostly present

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38.	*Caesalpinia bonduc* (Linn.) Roxb.	Katkaranja	Caesalpinaceae	Seed: colic pain, chronic fever, leprosy, ulcer, biles	Rare present
39.	*Cassia fistula* Linn.	Amaltash	Caesalpinaceae	Fruits laxative: blood purification, snake bite; Leaves: skin disease.	Seldom present
40.	*Cassia tora* (Linn.) C.	Chirota	Caesalpinaceae	Seed: ringworm, skin disease; Root: snake bite.	Mostly present
41.	*Cathranthus roseus* (Linn.) G. Don	Sadabahar	Apocynaceae	Plant: diabetes, sore throat, laryngitis & chest complaints; Leaves: menorrhagia; Root: stomachic.	Oftenly present
42.	*Cestrum nocturnum* Linn.	Rat ki rani	Solanaceae	Plant extract: antispasmodic and epilepsy.	Seldom present
43.	*Chenopodium album* Linn.	Bathua	Amaranthaceae	Leaves and plant: relieve stomach pains, stimulant to spleen disorders.	Rare present
44.	*Cissus quadrangularis* Linn.	Harshankari/ Hadjora	Vitaceae	Stem: asthma; Roots: fractured bones; Shoots: burns wounds.	Rare present
45.	*Citrus limon* (Linn.) Burm. f.	Nimbu	Rutaceae	Fruits: stomachic, antiscorbic, scurvy in rheumatism & diarrhea.	Oftenly present
46.	*Cleome viscosa* Linn.	Hurhur	Cleomaceae	Leaves: wounds ulcers, earache, seeds carminative, antihelmintic; Seeds: chronic painful joints.	Constantly present
47.	*Clitoria ternatea* Linn.	Aparajita	Fabaceae	Root bark: diuretic, swollen joints.	Seldom present
48.	*Coccinia indica* Wight & Arn.	Kunduru	Cucurbitaceae	Leaf & Root juice: diabetes, antiseptic & applied on skin eruptions; Plant: gonorrhea, bronchitis.	Rare present
49.	*Cocos nucifera* Linn.	Nariyal	Arecaceae	Fruit : diuretic, cold effects; Coconut water: stomachic redues heart burn, acidity of the stomach.	Seldom present
50.	*Coleus aromaticus* Benth.	Patharajwain	Lamiaceae	Leaves: cough, sore throats & nasal congestion.	Rare present
51.	*Commelina benghalensis* Linn.	Kanchara/ Kanuraka	Commelinaceae	Plant : leprosy, diuretic, febrifuge and anti inflammatory.	Constanly present
52.	*Coriandrum sativum* Linn.	Dhaniya	Apiaceae	Fruit: diuretic, colic, abdominal pain; Seed: ulcer.	Rare present
53.	*Costus speciosus* (Koen) Smith	Kuyukand	Zingiberaceae	Rhizome: fever, dropsy, cholera, bronchial asthma, rheumatism, haemorrhage.	Rare present
54.	*Crinum latifolium* Linn.	Sudarshan	Amaryllidaceae	Bulbs: rubefacient in rheumatism, piles and abscesses to cause suppuration, Leaf juice: earache.	Seldom present
55.	*Croton tiglium* Linn.	Jamalghota	Euphorbiaceae	Seed: asthma, bronchitis, paralysis, gout.	Mostly present
56.	*Cucurbita maxima* (Duch.) ex Lam.	Kaddu	Cucurbitaceae	Seeds: diuretic; Fruit: antiseptic on burns, boils & swellings.	Seldom present
57.	*Curcuma longa* Linn.	Haldi	Zingiberaceae	Termeric: stomachic, blood purifier, vermicide, antiseptic, carminatiue, diarrhoea, intermittent fevers, dropsy, jaundice, liver disorders and urinary troubles, foul ulcers.	Rare present
58.	*Cuscuta reflexa* Roxb.	Amar bel	Convolvulaceae	Plant: antiseptic; Seeds: carminative & astringent.	Seldom present
59.	*Cynodon dacytlon* (Linn.) Pers.	Doob/Dubi	Poaceae	Root: dropsy & secondary syphilis, root infection in bleeding piles & in	Constantly present

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				gleet; Plant juice: antisepitc applied on wounds. chronic diarrhoea dysentery, (epilespsy, insanity); Rhizomes: genito urinary disorders.	
60.	*Cypersu iria* Linn.	Motha/Dilla	Cyperaceae	Astringent and stomachache.	Seldom present
61.	*Cyperus rotundus* Linn.	Nagarmotha/ Motha	Cyperaceae	Plant: nausea, fever, inflammation, pain reduction and muscle relaxation Tubers: wounds, bruises, carbuncles	Oftenly present
62.	*Dalbergia sissoo* Roxb.	Shisham	Fabaceae	Leaves stimulant, gonorrhea; Root & wood: astringent, alternative used in leprosy, boils, eruptions of the skin & to allay vomiting.	Oftenly present
63.	*Datura alba* Ness	Safed dhatura	Solanaceae	Leaves: boils & sores; Seeds oils: antimicrobial activity.	Oftenly present
64.	*Datura metel* Linn.	Kala dhatura	Solanaceae	Leaves: smoked as an antispasmodic in asthma, whooping cough, brono-chitis; Fruits: to check dandruff & falling of the hair.	Rare present
65.	*Delonix regia* (Boj. Ex Hook). Raf.	Gulmohar	Caesalpinaceae	Bark : rheumatism.	Mostly present
66.	*Eclipta alba* (Linn.) Hassk.	Bhringraja	Asteraceae	Plant: hepatic & spleen enlargements sking troubles, jaundice; Root: antiseptic to ulcers.	Seldom present
67.	*Eichhornia crassipes* (Mart.) Solms	Jalkumbi	Pontederiaceae	Flower: skin trouble.	Oftenly present
68.	*Emblica officinalis* Gaetrn.	Amla	Euphorbiaceae	Fruit: richest source of Vitamin C, diuretic & laxative, fevers, vomiting indigestion troubles; Dried fruit: diarrhea, dysentery, hemorrhage in anemia, jaundice etc.	Oftenly present
69.	*Ervatamia coronaria* (Jacq.) Stapf	Chadni	Apocynanceae	Roots: as anodyne, chewed for relief in toothache; Leaves: ophthalmia, colling the wounds; Flower: burning sensation of eyes and in skin irritation.	Constantly present
70.	*Eucalyptus globulas* Labill.	Neelgiri	Myrtaceae	Leaves: asthma, influenza & bronch-itis; Oil: muscular pains, antiseptic, infections of the upper respiratory tract & certain skin diseases, ointments for burns; Root: purgative.	Oftenly present
71.	*Euphorbia hirta* Linn.	Dudhiya	Euphorbiaceae	Plant: colic pains, gentio-urinary tract; Plant latex: nervousness diseases, dropsy, worm infection & skin diseases.	Constantly present
72.	*Euphorbia neriifolia* Linn.	Sehund	Euphorbiaceae	Plant juice: earache, ophthalmia, asthma.	Oftenly present
73.	*Ficus benghalensis* Linn.	Bargad	Moraceae	Leaves : antimicrobial, removes mouth ulers; Latex: antiseptic applied on wounds; Bark: dysentery & diabetes, Buds: diarrhoea.	Seldom present
74.	*Ficus religiosa* Linn.	Peepal/Vat	Moraceae	Bark : gonorrhea, scabies.	Oftenly present
75.	*Gossypium arboreum* Linn.	Kapas	Malvaceae	Roots : fevers; Seed: gonorrhea, gleet, cystitis, catarrh in consumption; Leaves: blood dysentery.	Rare present

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76.	*Gymnema sylvestre* R. Br.	Gummar	Asclepiadaceae	Extracts of Gymnema: hyperglycemia, obesity, high cholesterol levels, anemia and digestion.	Seldom present
77.	*Helianthus annuus* Linn.	Surajmukhi	Asteraceae	Plant: antiseptic; Seeds: diuretic, expectorant given in pulmonary infections; Leaves : fever due to malaria parasite.	Rare present
78.	*Heliotropium annuus* Linn	Hattajurie/Hattasura	Boraginaceae	Leaves: wounds, skin ulcers, furuncles, eye drop for conjunctivities and poultice.	Oflenly present
79.	*Hibiscus rosa-sinensis* Linn.	Gurhal	Malvaceae	Leaves: applied to boils, bronchial catarrh with ghee in menorrhagia; Leaves: fever; Root: gonorrhea, cough, fevers.	Mostly present
80.	*Ipomoea aquatica* Forssk.	Kalmisag	Convolvulaceae	Plant: piles, nervous disorders; Buds: ringworm.	Seldom present
81.	*Ixora coccinea* Linn.	Kangan	Rubiaceae	Roots: nausea, hiccups, and anorexia, mouthwash and gargles for sore throal; Flowers: dysentery and leucorrhea, hypertension, amenorrha and irregular menstruation, sprains, exema, boils, wonders and skin ulcers; Fresh Leaves and Stems.	Oflenly present
82.	*Ixora congesta* Linn.	Jarum jarum	Rubiaceae	Leaves: skin complaints and wounds Roots: sore throat and flowers: hypertension.	Oflenly present
83.	*Jasminum sambac* (Linn.) Aiton	Mogra	Oleaceae	Leaves: treat gallstones; Root: diabetes mellitus, headaches; Flower: antidepressant; Jasmine oil: addiction, depression, nervousness, coughs, relaxation and tension.	Seldom present
84.	*Jatropha curcas* Linn.	Ratanjot	Euphorbiaceae	Latex: bleeding, wounds, piles, scabies, ringworm, itch & decayed teeth; seed oil: skin disease & bleeding wounds.	Rare present
85.	*Lantana camara* Linn.	Raimunjya/ Dhani-dadia	Verbenaceae	Leaves: rheumatism.	Mostly present
86.	*Lawsonia inermis* Linn.	Henna/Mehandi	Lythraceae	Leaves: skin disease, boils, inflammations, sore throat.	Rare present
87.	*Leucas aspera* Willd..	Chhota halkusa/ Gophaa	Lamiaceae	Leaves: boils, blisters, psoriasis, skin eruptions & painful swellings; Flower: cough, & cold, antibacterial activity.	Oflenly present
88.	*Leucas cephalotes* (Roth) Spreng	Dhurpisag/ Gumma	Lamiaceae	Plant: diaphoretic, antiseptic; Flowers: cough & cold.	Seldom present
89.	*Lycopersicon esculentum* Mill.	Tomato	Solanaceae	Fruits: antimicrobial activity; Fruits diurectic diseases, stomatitis, hepatitis and general debility. Sliced Fruits: burns, scaled and sunburn; Root: toothache.	Seldom present
90.	*Mangifera indica* Linn.	Aam	Anacardiaceae	Leaves: antimicrobial activity, Fruits: diurectic given in haemorrhaids of uterus, lungs & intestines: Unripe fruits: ophtalmia & skin diseases for blood purification, fruit rind stimulant, tonic & used in somach debility.	Oflenly present

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91.	*Melilotus indicus* (Linn.) All.	Banmethi	Fabaceae	Leaves: swellings. astringent, nactrotic; Seed: bowel complaints & diarrhea.	Seldom present
92.	*Mentha piperita* Linn.	Pudina	Lamiaceae	Herb aromatic, stimulant, stomachic & Carmnative, vomiting in headache, sprain, stomachache, colic, constipation.	Mostly present
93.	*Michelia champaca* Linn.	Champa	Mangnoliaceae	Bark : stimulant, expecorant & astringent Roots: dyspepsia fever, ; renal diseases; Leaf juice: colic; Seeds & fruits: antiseptic.	Seldom present
94.	*Mimosa pudica* Linn.	Lajjavanti	Mimosaceae	Roots: piles, conjunctivities, diarrhea, amoebic dysentery, gynecological disorders, skin diseases, bronchitis, general weakness and impotence; Leaves : vaginal diseases, wounds, ulcers, fistula, scrofula, hydrocele, whole plant: edema and rheumatism.	Rare prsent
95.	*Momordica charantia* Descourt	Karela	Cucurbitaceae	Fruit pulp and seeds: skin diseases, vermifuge and fever; Leavers: galactogogue antibacterial, antibiotic, antidiabetic, anti-inflammatory, antimicrobial, antileukemic, antimutagenic, antioxidant, antitumor, antiulcer, antiviral astringent, carminative, stomachic; Roct:: astringent.	Oftenly present
96.	*Moringa oleifera* Linn.	Munga	Moringaceae	Seed: abdominal tumors: Root: dropsy; Leaves: sores, headaches.	Oftenly present
97.	*Morus alba* Linn.	Shahtoot	Moraceae	Leaves: throat sores, dyspepsia & melancholia. Root: anthelmintic, purgative astringents, diarrhea; Fruit: high fever.	Rare present
98.	*Murraya koenigii* (Linn.) Sprengel	Methaneem	Rutaceae	Leaves & Root: stomachic, carminative, dysentery, diarrhea & check vomiting; Bark: skin eruptions.	Oftenly present
99.	*Musa paradisiace* Linn.	Kela	Musaccae	Banana fruit: intestinal disorders, uremia, nephritis, hypertension & other vascular diseases, Root: anthelmintic; Flowers: astringent, cough & cold: Stem: dyspepsia & stomach disorders; Root: blood diseases; Leaves: antiseptic.	Rare present
100.	*Nerium oleander* Linn.	Kaner	Apocynaceae	Whole plant: anticancer properties; Leaves: scabies and to reduce swellings; Roots: cardiac asthma, chronic stomache, joint pains, leprosy and ulcers.	Seldom present
101.	*Nyctanthes arbor-tristis* Linn.	Parijat/ Harsinghar	Oleaceae	Leaves: antiseptic, fevers, rheumatism; Seeds: scurfy affections of scalp.	Seldom present
102.	*Nymphaea alba* Linn.	Gul Nilofar	Nymphaeaceae	Rhizomes: diarrhea, Flowers: stomach disorders.	Oftenly present
103.	*Nymphaea nouchali* Burm.f.	Neelpadma	Nymphaeaceae	Powderd rhizomes: diarrhea, piles; Leaf: fevers; Flower; narcotic, seed stomachic.	Oftenly present
104.	*Ocimum basilicum* Linn.	Bantulsi	Lamiaceae	Plant: infusions in cephalalgia, gouty joints, gorgle in foul breeth leaf	Seldom present

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				juice : nacrotic, cold effect, seeds : diaphorte, piles	
105.	*Ocimum sanctum* Linn.	Tulsi	Lamiaceae	Leaf juice: chronic fever, haemorrhage dysentery & dyspepsia, check vomiting throat congestion, liver disorder, Leaf extract in cold effects; Flowers: cough, headache; Seeds: fever, stimulant in colitis.	Constantly present
106.	*Opuntia dillinii* (Ker Gawl) Ham	Nagphane	Cactaceae	Ripe Fruits: urine red, gonorrhea; Leaf pulp: ophthalmia; Flowers: bronchitis and asthma. Plant: heating, cures tumours, leucoderma, stoma chic, carminative, antipyretic, burning, leucoderma, urinary complaints, tumours, loss of consciousness, piles, inflemmations, anaemia, ulcers and the enlargement of the spleem.	Seldom present
107.	*Oxalis corniculata* Linn.	Khatti-Butti/ Tinpania bhaji	Oxalidaceae	Plant juice: dyspepsia, piles, tympanitis, cooling, stomachic, antiscorbutic, appetizing, fevers, dysentery, scurvy, for removing warts, in intoxication; Leaf extract: gargling.	Oftenly present
108.	*Passiflora caerulea* Linn.	Jhumkalata	Passifloraceae	Fresh or Dried Leaves: insomnia, hysteria, and epilepsy Flower: stress and anxiety.	Rare present
109.	*Portulaca oleracea* Linn.	Lunia/Badi noni	Portulacaceae	Whole plant: tumor, inflammations, Jundice, diabetes burns, headache, burning sensation, vomiting, dysentery, skin diseases, and stomatitis.	Mostly present
110.	*Phoenix acaulis* Roxb.	Chhind	Arecaceae	Grass: fever.	Oftenly present
111.	*Phragmites karka* (Cav.) Trin. ex Steud,	Bagnarri	Poaceae	Root: fractured bones	Rare present
112.	*Phyllanthus niruri* Linn.	Bhuiaonla	Euphorbiaceae	Plants: diuretic properties (genito - urinary tract); Fresh Leaves: jaundice and intermittent fever; Roots: jaundice	Constantly present
113.	*Pinus roxburghii* Sargent	Chir	Pinaceae	Plant: antiseptic, expectorant given in chronic bronchitis and applied in haemorrhages of nose.	Rare present
114.	*Pistia stratiotes* Linn.	Jalmandavi	Araceae	Leaves: antiseptic, anti dysenteric, good in eye wash, ring worm infection; Leaf juice: eczema, leprosy, ulcers, piles and syphilis, cough, asthma.	Rare present
115.	*Pongamia pinnata* (Linn.) Pierre	Karanj	Fabaceae	Seeds: skin diseases & rheumatism; Fresh Bark: bleeding Piles; Roots: closing fistulous sores & for cleansing foul ulcers & sexual diseases; Flower: diabetes.	Oftenly present
116.	*Prunus amygdalus* (Mill.) D.A. Webb	Badam	Rosaceae	Seeds: respiratory trouble, urinogenital system, diabetes.	Seldom present
117.	*Psidium guajava* Linn.	Amrood/Jam	Myrtaceae	Leaves: cholera, to check vomiting in diarrhea, astringent; Shoot & leaf infusion: antispasmodic, body pain, nephritis, locally applied on swelling & rheumatism, Flowers: bronchitis,	Oftenly present

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				eye sores, bleeding gums & colitis; Fruits: colic and bleeding gums.	
118.	*Punica granatum* Linn.	Anar	Lythraceae	Fruit: cough, throat troubles & cold effect, dyspepsia, diarrhea & dysentery; Powderd Flower buds: bronchitis.	Seldom present
119.	*Putarnjiva roxburghii* Wall.	Putrajiva	Putranjivaceae	Leaves: cold & fever in rheumatism; Crushed Leaves: swollen throut.	Rare present
120.	*Rauvolfia serpentina* (Linn.) Benth. ex Kurz	Sarpagandha	Apocynaceae	Root: intestinal disorders, cholers, cholera, colic; Leaf juice: inopacity of corna, Roots: bowels & fever.	Seldom present
121.	*Rhoeo discolor* (L'Her.) Hance		Commelinaceal	Flowers & Leaves : Cough cold, coughing phlegm with blood whooping cough. Nose bleed, Bacillary dysentery, blood in the stool.	Seldom present
122.	*Ricinus communis* Linn.	Arand	Euphorbiaceae	Oil: acule diarrhea, constipation, conjunctivities. Leaves: headache and as poultice for boils.	Seldom present
123.	*Rosa centifolia* Linn.	Gulab	Rosaceae	Flower: epilepsy & insomnia; diarrhea.	Mostly present
124.	*Saccharum spontaneum* Linn.	Kash	Poaceae	Plant: burning sensation, phthsis, vesical cakuli, blood diseases, halmorrhagic, diathesis, biliousness.	Oftenly present
125.	*Saccharum officinarum* Linn.	Ganna	Poaceae	Powdered sugar: gonorrhea and vaginal discharges; spontaneum Linn. Pulped sugar cane: dress wounds and the cane for splints for broken bones; Plant juices: abdominal tumors, catarrh, arthritis, bedsores, boils, inflammation, wounds, refrigerant, and stomachic.	Seldom present
126.	*Sansevieria trifasciata* Prain.	Snake Plant	Liliaceae	Leaves: herpes zoster, Leaves juice: earache.	Seldom present
127.	*Saraca indica* Linn.	Ashok	Fabaceae	Dried Bark: astringent, uterine disorders, especially in menorrhagia, leucorrhoea, piles and dysentery; Dried Flowers: diabetes; Seeds: urinary discharges.	Oftenly present
128.	*Sesamum indicum* Linn.	Til/Tilli	Pedaliaceae	Leaves, Seeds & Flower: carminative, diuretic wound healer.	Rare present
129.	*Sida cordifolia* Bunm.f.	Bariara	Malvaceae	Plant: pulmonary tuberculosis; Leaves rheumatic pains, ulcers, swellings & elephantiasis.	Mostly present
130.	*Sida cordifolia* Linn.	Bariyari	Malvaceae	Seeds: improving sexual strength; Root: fever.	Seldom present
131.	*Solanum nigrum* Linn.	Makoy	Solanaceae	Plant: dropsy enlargement of the liver and jaundice, fever; Leaf juice: inflammation of the kidneys and bladder, heart diseases, swollen and painful testicles, ulcers and skin diseases.	Rare present
132.	*Solanum surattense* Burm.F.	Kantakari	Solanaceae	Roots: cough, asthma, chest pain; Stem, Flower & fruits: carminative, relieve burning sensation in soles & fect; Seeds asthma, cough, diuretic,	Rare present

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				fever; Leaf juice rheumatism, dengue fever, acute bronchitis.	
133.	*Solanum xanthocarpum* Schrad. & Wendl.	keteli/ Bhatakateri	Solanaceae	Plant: carmiative & diuretic, fevers, coughs, asthma, dropsy, gonorrhea, pain in the chest & heart diseases; Fruit: jaundice, tied to organ to reduce the swellings; Dried Roots: cough, asthma pain in chest & fever.	Mostly present
134.	*Sterculia foetida* Linn.	Junglibadam/ Kalumpang	Sterculiaceae	Seeds: purgativer; Leaves: skin eruptions, difficult labor; Fruit: astringent; Oil: pruritic conditions.	Rare present
135.	*Syzygium cumini* (Linn.) Skeets.	Jamun	Myrtaceae	Fresh Bark juice: diarrhea: Seed: diabetes; Bark: sore throats, bronchitis, asthma, ulcers and dysentery.	Seldom present
136.	*Tagetes erecta* Linn.	Genda	Asteraceae	Leaves & Fruit: Piles, fever, inflammation, ulcer, menorrhagia.	Constantly present
137.	*Tamarindus indica* Linn.	Imali	Caesalpinaceae	Leaves: inflammatory swellings & ringworm; Furit: inflammatory illnes' Fruits: anthelmintic.	Seldam present
138.	*Tecoma stans* (Linn.) Juss. ex Kunth	Piliya	Bignoniaceae	Roots: powerful diuretic, vermifuge.	Seldom present
139.	*Tectona grandis* (Linn.) F.	Sagaun	Verbenaceae	Wood: antiseptic, dyspepsia, swellings, Bark: astringent, scabies, Flowers & seeds: diuretic; anthelmintic.	Rare present
140.	*Tephrosia purpurea* (Linn.) Pers.	Sharpunkha	Fabaceae	Plant: liver, tumors, asthma, leprosy; Root: diuretic, cure diarrhea; Leaves: piles and gonorrhea.	Oftenly present
141.	*Terminalia arjuna* Bedd.	Arjun	Combretaceae	Bark: astringent, cardiac stimulant.	Seldom present
142.	*Thevetia peruviana* Juss.	Pila kaner	Apocynaceae	Seed: dropsy, rheumatism; Root and Root Bark: skin disease, cuts, wounds, tumors.	Mostly present
143.	*Thuja occidentalis* Linn.	Vidyapalti	Cupressaceae	Leaves: fever, gout, coughs. Rheumatism, scurvy.	Seldom present
144.	*Tinospora cordifolia* (Thunb.) Micrs	Giloe	Menispermaceae	Plant: diuretic, stimulant rheumatism, urinary diseases, skin diseases, piles, bronchitis & jaundice; Roots & Stem: fevers chronic diarrhea, chronic dysentery and jaundice.	Rare present
145.	*Trapa natans* Linn. Linn.	Singhara	Trapaceac	Dried Seed: cooling, stomachic.	Mostly present
146.	*Tridax procumbens* Linn.	Khal-muriya/ Taj-muriya	Asteraceae	Leaves: dysentery, diarrhea, antiseptic.	Mostly present
147.	*Trigonella foenum-graecum* Linn.	Methi	Fabaceae	Seeds: hard boils & emollient, intestinal inflammation; Flower: hemorrhoidal, tumors, rectal injection for dysentery, diarrhea, bilious and inflammatory colic.	Rare present
148.	*Vitis vinifera* Linn.	Grapes	Vitaceae	Fresh grapes: stomachic, diurectic, demulcent and cooling. Leaves : astringent diarrhera, young bunnches: skin infections.	Rare present
149.	*Withania somnifera* (Linn.) Duna	Ashwagandha	Solanaceae	Leaves: skin lesions, ulcers, boils and swelling to reduce pus formation;	Rare present

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				Roots: hiccup, cough, dropsy, rheumatism & female disorders.	
150.	*Woodfordia fruticose* (Linn.) Kurz	Arishtas/Dhai	Lythraceae	Dried flowres: bowel, haemorrhages, seminal weakness, ulcers; Leaves skin disease, conjunctivitis.	Rare present
151.	*Zingiber officinale* Roscoe	Adarak	Zingiberaceae	Rhizome: stimulant, carminative, dyspepsia and flatulent colic, rheumatism, piles, pulmonary, catarrhal diseases, dropsy, coughs, asthma, headache & toothache.	Rare present
152.	*Ziziphus mauritiana* Linn.	Ber	Rhamnaceae	Fruit: chest troubles; Seeds; diarrhea & stop vomiting.	Mostly present

determining species for the physiognomy of the community of the Durg Nagar Nigam Area.

Thus it was very evident that the flora of the study area showed a great diversity and a variation in seasonal aspects, so for as the medicinal plants are concerned. The above plants are though used extensively by the people yet the methods are crude and need proper standardization for effective and scientific treatment. Again the active principles of the alkaloids of these plants have yet not been worked out and there is a great need for further research in this area so that these cures can be exploited at international level. Therefore there is a great need for the conservation *in situ* and *ex situ* of the rare medicinal plant species; so that these do not become rare or endangered because of over exploitation. Awareness to the local people, about the conservation and maintenance may also help to evolve an effective indigenous technology for the welfare of the state.

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Combining Ability and Heterosis for Seed Yield, Yield Components and Oil Content in Yellow *Sarson* (*Brassica campestris* var. yellow sarson)

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Twenty diverse genotypes of yellow sarson were crossed with two varieties of yellow sarson in a line x tester mating design to produce 40 hybrids. The 40 crosses and their 22 parents were evaluated for seven quantitative traits for investigating general and specific combining ability and heterosis over better-parent and standard variety (NDYS-2). Non-additive gene action played predominant role in the expression of seed yield, its components and oil content. The lines, MYSL-2003-2, NDYS-9503, NDYS-128, NRCY-7, MYSL-2004, NRCY-5, RAUDYS-89-1 and NDYS-141 emerged as good general combiner parents for seed yield and some of its components. Based on *sca* effects, the most desirable crosses for seed yield and its components were NDYS-141 x YST-151, NDYS-128 x NDYS-2, BIO-YS-1 x NDYS-2, NDYS-9503 x NDYS-2, NRCY-5 x YST-151, NDYS-7 x NDYS-2, RYSK-05-1 x YST-151 and NDYS-17 x YST-151. The crosses, NDYS-9503 x NDYS-2, MYSL-2003-2 x NDYS-2, NDYS-128 x NDYS-2 and NRCY-7 x NDYS-2 showed very high heterosis for seed yield and some of the yield contributing traits.

Keywords : Combining ability, Heterosis, Yellow sarson.

INTRODUCTION

Rapeseed-mustard contributes about 32% of the total oilseed production in India as the third largest indigenous oilseed crop after groundnut and soybean. Yellow *sarson* (*Brassica campestris* var. yellow sarson) is one of the important crops in the rapeseed-mustard group. In spite of possessing superior oil content and quality, yellow *sarson* is characterized by low yield potential. For enhancing the yield potential, the development of genotypes possessing high seed yield and oil content through reshuffle of genes by hybridization involving chosen parents and exploitation of heterosis of promising crosses appear essential. The present investigation was under taken for identification of potential parents and crosses on the basis of combining ability and heterosis for exploitation in breeding programmes aimed at developing high yielding varieties of yellow sarson possessing high oil content.

MATERIAL AND METHODS

Two varieties of yellow sarson, NDYS-2 and YST-151 were crossed as testers with 20 diverse lines, namely, NDYS-128, NDYS-141, NDYS-116-1, NDYS-9503, NDYS-9506, NDYS-9508, RYSK-05-1, BINOY, MYSL-2003-2, NDYS-7, NDYS-17, NDYS-51, MYSL-2005, NDYS-921, NRCY-5, NRCY-7, SSK-92-13, MYSL-2004, RAUDYS-81-9 and BIO- YS-1. The experimental material comprising of 20 lines, two testers and their 40 crosses in line x tester design was evaluated in randomized complete block design with three replications at Pilikothi Farm of T.D.P.G. College, Jaunpur, during *rabi* season of 2006-07. Each genotype was sown in single row plot of 3 m length with inter- and intra-row spacings of 30 cm and 10 cm, respectively. The observations were recorded on five randomly selected competitive plants in each plot for seven quantitative traits *viz.* days to maturity, siliquae per plant, seeds per siliqua, 1000-seed weight, seed yield per plant, harvest-index and oil content. The mean values of each genotype were subjected to combining ability analysis (Kempthorne, 1957) and estimation of heterosis as per standard procedure.

RESULTS AND DISCUSSION

The analysis of variance for combining ability (Table 1) revealed that mean squares due to lines were significant for all the characters except 1000-seed weight and oil content, while the mean squares due to testers were significant only for 1000-seed weight showing involvement of additive gene action in these cases. The highly significant variances due to line x tester interactions for all the seven characters under study indicated importance of non-additive gene action. Predominance of non-additive gene action was evident by greater than one values of average degree of dominance resulting due to higher specific combining ability (*sca*) variance as compared to general combining ability (*gca*) variance for

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Table 1 : Analysis of variance for combining ability and estimates of components of genetic variance for eleven characters in yellow sarson.

Source of variation	d.f.	Days to maturity	Siliquae per plant	Seeds per siliqua	1000- seed weight (g)	Seed yield per plant (g)	Harvest index	Oil content (%)
Lines	19	62.45**	1169.70**	106.80**	0.58	11.48**	25.75**	8.45
Testers	1	25.21	12.55	0.75	3.23*	0.19	3.75	0.77
Lines x Testers	19	9.52**	116.80**	12.82**	0.65**	2.64**	4.87**	4.72**
Error	122	1.88	2.22	1.48	0.01	0.23	0.66	0.05
Components of genetic variance								
σ^2 *gca*		0.29	5.72	0.51	0.001	0.05	0.11	0.02
σ^2 *sca*		8.02	101.66	9.15	0.49	1.17	2.76	1.39
$(\sigma^2 sca/2\ \sigma^2 gca)^{1/2}$		3.72	2.98	2.99	15.65	3.49	3.48	6.05
σ^2 A		1.17	22.87	2.04	0.002	0.19	0.46	0.08
σ^2 D		32.08	406.65	36.58	1.96	4.66	11.06	5.57

* Significant at 5% probability level,
** Significant at 1% probability level.

all the characters. This indicated scope of improvement in these traits in yellow *sarson* through heterosis breeding. Gautam (2008) and Verma *et al.* (2009) have also reported preponderance of non-additive gene action for seed yield, 1000-seed weight, oil content, siliquae per plant and seeds per siliqua in yellow *sarson*.

The estimates of general combining ability (*gca*) effects showed that none of the parents was found to be good general combiner for all the traits. Eight lines, MYSL-2003-2, NDYS-9503, NDYS-128, NRCY-7, MYSL-2004, NRCY-5, RAUDYS-89-1 and NDYS-141 were identified as good general combiners for seed yield per plant and two to four other characters. The best general combiner for seed yield per plant, MYSL-2003-2 also emerged as good general combiner for 1000-seed weight, seeds per siliqua and siliquae per plant. None of the testers were found to be good general combiners for seed yield per plant.

Out of 40 crosses, two cross combinations for days to maturity, 12 for siliquae per plant, seven for seeds per siliqua, 16 for 1000-seed weight, eight for seed yield per plant, six for harvest-index and 15 for oil content showed significant specific combining ability (*sca*) effects in desirable direction. The best five crosses exhibiting significant and desirable *sca* effects for seven characters are listed in Table 2. Eight crosses, NDYS-141 x YST-151, NDYS-128 x NDYS-2, BIO-YS-1 x NDYS-2, NDYS-9503 x NDYS-2, NRCY-5 x YST-151, NDYS-7 x NDYS-2, RYSK-05-1 x YST-151 and NDYS-17 x YST-151 showed positive and significant specific combining ability effects for seed yield per plant along with significant *sca* effects in desirable direction for three or more other component characters. It was observed that crosses showing significant and positive sca effects resulted from all sort of combinations of *gca* effects of parents ranging from high x high to low x low general combiner parental combinations. Therefore, there was no particular relationship between the positive and significant *sca* effects of the crosses with the *gca* effects of their parents.

Based on heterosis over better-parent (heterobeltiosis) and standard variety (standard heterosis), the five best crosses were identified for each character (Table 2). A wide range of variation was observed in the estimates of heterobeltiosis and standard heterosis for all the seven characters under study. The very high positive mean heterosis for seed yield per plant was found to be 45.87% and 55.78% in case of heterobeltiosis and standard heterosis, respectively. Out of 40 crosses, the crosses exhibiting positive and significant heterobeltiosis and standard heterosis for seed yield per plant were 34 and 39, respectively. Similarly, high manifestation of heterosis of both types was observed for remaining six characters also. Tiwari (2007), Gautam (2008) and Verma *et al.* (2009) have also reported high manifestation of heterobeltiosis and standard heterosis for seed yield and its components in yellow sarson. The cross, NDYS-9503 x NDYS-2 showed highest heterobeltiosis (120.78%) and standard heterosis (120.78%) for seed yield per plant along with positive and significant heterosis of both types for 1000-seed weight, harvest-index and siliquae per plant. The cross, MYSL-2003-2 x NDYS-2 showed very high positive heterobeltiosis (114.29%) and standard heterosis (114.29%) for seed yield per plant besides having significant heterosis of both types in desirable direction for 1000-seed weight, siliquae per plant and early maturity. NDYS-128 x NDYS-2 had very high estimates of both types of heterosis in desirable direction for early maturity, seeds per siliqua and seed yield per plant (BP = 110.39%, SP = 110.39%). Similarly, NRCY-7 x NDYS-2 had significant positive heterobeltiosis and standard heterosis for seed yield per plant (BP = 81.82%, SP = 81.82%), oil content and siliquae per plant. These four crosses exhibiting very high mean performance and manifestation of heterobeltiosis and standard heterosis for seed yield per plant and several important yield components including early maturity, merit special attention for exploitation in breeding programme aimed at isolating superior segregants for deriving high yielding and early maturing varieties of yellow sarson.

In the present study, seed yield and its contributing traits like siliquae per plant, seeds per siliqua, 1000-seed weight,

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Table 2: Best five crosses for desirable specific combining ability (*sca*) effects, heterobeltiosis and standard heterosis for seed yield, its components and oil content in yellow sarson.

Characters	Heterosis over standard variety	Heterosis over better parent	*Sca* effects
1	2	3	4
Days to maturity	RAUDYS-89-1 x NDYS-2 (-7.46)** NDYS-9503 x NDYS-2 (-6.87)** BIO-YS-1 x YST-151 (-6.87)** BINOY x YST-151 (-6.57)** NDYS-9508 x YST-151 (-6.27)**	SSK-92-13 x NDYS-2 (-13.93)** NDYS-9503 x NDYS-2 (11.36)** NDYS-51 x NDYS-2 (-11.17)** NDYS-51 x YST-151 (-11.11)** SSK-92-13 x YST-151 (-10.93)**	NDYS-9503 x NDYS-2 (-3.13)** SSK-92.-13 x NDYS-2 (-2.29)**
Siliquae per plant	MYSL-2004 x NDYS-2 (29.28)** MYSL-2004 x YST-151 (26.14)** NRCY-5 x NDYS-2 (25.15)** NDYS-9506 x YST-151 (22.04)** NDYS-921 x YST-151 (21.75)**	NDYS-9506 x YST-151 (71.73)** NRCY-5 x YST-151 (67.76)** NDYS-9508 x YST-151 (61.13)** SSK-92-13 x YST-151 (58.06)** MYSL-2005 x YST-151 (56.51)**	NDYS-141 x YST-151 (9.32)** BIO-YS-1 x NDYS-2 (7.54)** NDYS-9506 x YST-151 (6.07)** SSK-92-13 x YST-151 (6.01)** NDYS-51 x NDYS-2 (5.59)**
Seeds per siliqua	NDYS-141 x YST-151 (10.31)** NDYS-128 x NDYS-2 (10.08)** MYSL-2004 x NDYS-2 (6.57)**	NDYS-141 x YST-151 (23.60)** MYSL-2004 x YST-151 (16.97)** NDYS-128 x NDYS-2 (10.08)** MYSL-2004 x NDYS-2 (6.57)**	NDYS-128 x NDYS-2 (3.15)** NDYS-17 x YST-151 (2.59)** NDYS-9506 x YST-151 (2.52)** MYSL-2005 x YST-151 (1.82)** RAUDYS-89-1 x NDYS-2 (1.78)**
1000-seed weight	SSK-92.-13 x YST-151 (44.50)** RAUDYS-89-1 x YST-151 (42.49)** NDYS-7 x NDYS-2 (39.14)** MYSL-2003-2 x YST-151 (38.66)** MYSL-2003-2 x NDYS-2 (37.42)**	NDYS-7 x NDYS-2 (39.14)** NDYS-51 x NDYS-2 (23.44)** NDYS-17 x NDYS-2 (21.62)** SSK-92-13 x YST-151 (20.22)** RAUDYS-89-1 x YST-151(18.55)**	NRCY-7 x YST-151 (0.68)** RAUDYS-89-1 x YST-151 (0.53)** BINOY x YST-151 (0.50)** NDYS-9503 x NDYS-2 (0.45)** NDYS-7 x NDYS-2 (0.40)**
Seed yield per plant (g)	NDYS-9503 x NDYS-2 (120.78)** MYSL-2003-2 x NDYS-2 (114.29)** NDYS-128 x NDYS-2 (110.39)** MYSL-2003-2 x YST-151 (100.00)** NRCY-5 x YST-151 (92.21)**	NDYS-9503 x NDYS-2 (120.78)** MYSL-2003-2 x NDYS-2(114.29)** NDYS-128 x NDYS-2 (110.39)** NRCY-7 x NDYS-2 (81.82)** MYSL-2004 x NDYS-2 (76.62)**	NDYS-141 x YST-151 (1.26)** NDYS-128 x NDYS-2 (1.17)** BIO-YS-1 x NDYS-2 (1.14)** NDYS-9503 x NDYS-2 (0.94)** NRCY-5 x YST-151 (0.79)**
Harvest-index	SSK-92-13 x NDYS-2 (26.09)** NDYS-116-1 x YST-151 (25.36)** NDYS-141 x NDYS-2 (22.59)** NDYS-141 x YST-151 (21.14)** NDYS-17 x NDYS-2 (21.14)**	SSK-92-13 x NDYS-2 (21.32)** NDYS-9506 x YST-151 (21.23)** NDYS-9506 x NDYS-2 (18.08)** RYSK-05-1 x YST-151 (17.79)** NDYS-9503 x YST-151 (17.64)**	NDYS-116-1 x YST-151 (1.93)** SSK-92-13 x NDYS-2 (1.82)** NDYS-17 x NDYS-2 (1.46)** BINOY x YST-151 (1.31)** MYSL-2005 x NDYS-2 (1.22)**
Oil content (%)	NRCY-7 x NDYS-2 (10.13)** NDYS-51 x NDYS-2 (9.65)** NDYS-51 x YST-151 (8.93)** SSK-92-13 x NDYS-2 (8.93)** MYSL-2004 x YST-151 (8.69)**	SSK-92-13 x NDYS-2 (8.93)** NDYS-51 x NDYS-2 (8.52)** NRCY-7 x NDYS-2 (5.10)** MYSL-2005 x NDYS-2 (4.55)** NDYS-921 x NDYS-2 (3.99)**	NRCY-7 x NDYS-2 (1.66)** MYSL-2004 x YST-151 (1.60)** BIO-YS-1 x YST-151 (1.49)** NDYS-128 x YST-151 (1.20)** NDYS-17 x NDYS-2 (1.13)**

* Significant at 5% probability level,
** Significant at 1% probability level.

harvest-index and oil content were governed predominantly by non-additive gene action indicating the scope of genetic improvement in yellow *sarson* through exploitation of hybrid vigour. The crosses exhibiting very high heterosis for seed yield and yield components, namely, NDYS-9503 x NDYS-2, MYSL-2003-2 x NDYS-2, NDYS-128 x NDYS-2 and NRCY-7 x NDYS-2 could be used for commercial exploitation of heterosis in yellow sarson.

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Line x Tester Analysis in Bittergourd (*Momordica charantia* L.)

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A line x tester analysis studies in bitter gourd (*Momordica charantia* L.) was taken with five lines and three testers to estimate the combining ability effects and heterosis for ten biometrical traits over standard check of variety MC-13. Results of combining ability estimates revealed that the lines L_1 (MC 13) and L_2 (Panruti local) were observed as good general combiner for fruit yield per vine. The lines L_3 (Ayakudi local) and L_5 (Mithipagal) recorded negative *gca* effects for days to first female flowering and days to fruit maturity that could be utilized in breeding programme to develop bitter gourd hybrids with earliness. The lines L_1 and L_2 exhibited high *per se* values for most of the yield attributing characters. $L_3$and L_5 registered low values of *per se* for days to first female flowering and days to fruit maturity and high *per se* values for number of fruits per vine. Among the three testers T_1 (CO 1) and T_2 (VK 1 Priya) registered high mean performance for fruit length, cavity size index and individual fruit weight. The hybrids L_1 x T_3 (MC 13 x Arka Harit) and L_2 x T_2 (Panruti local x VK 1 Priya) registered high *per se* and *sca* for individual fruit weight and yield per vine. Combining ability estimates also revealed that additive x additive and additive x dominance type of interaction for days to female flowering, days to fruit maturity, number of fruits vine, fruit length, fruit size index, cavity size index individual fruit weight and yield per vine. Thus indicating the importance of heterosis breeding in bitter gourd. The cross L_1 x T_3 registered the highest heterosis over check variety MC 13 for fruit length, fruit size index and cavity size index. It is a product of good x good combiner. The hybrids L_1 x T_3 and L_2 x T_2 recorded the highest heterotic effects for number of fruits per vine and yield per vine over the check variety. The same hybrids also expressed higher *per se* for individual fruit weight and yield per vine. The crosses L_3 x T_2, L_5 x T_2, L_5 x T_3, and L_3 x T_3 recorded the highest heterosis for number of fruits per vine.

Keywords : Line x Tester analysis, Bitter gourd, Combining ability, Heterosis.

INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is one of the most important nutritious cucurbits highly valued for its medicinal use. The bitter principle momordicin, a bitter glucoside present in the fruits are used in the preparation of medicines particularly for the use of diabetics. Bitter gourd is a monoecious crop exhibited wide variability for several qualitative and quantitative characters and it does not suffer from inbreeding depression and its population structure seems that of inbreeders than outbreeders (Allard, 1960). Despite its wider variability limited attention has been paid for its improvement. The impulse of progress in crop improvement through plant breeding was propelled by a better understanding and an appropriate exploitation of heterosis. It is well known fact that heterosis breeding is a quicker and easier method of increasing yield, yield contributing and other economic characters. Any hybridization programme in a crop improvement work choice of suitable parents is a pre requisite. The common approach of selecting the parents on the basis of *per se* performance does not necessarily lead to fruitful results. Line x Tester analysis is one of the powerful tools developed by Kempthrone (1957) to assess the potentialities of the parents based on combining ability estimates.

MATERIAL AND METHODS

A field experiment was conducted at Department of Horticulture, Faculty of Agriculture, Annamalai University with five lines *viz.*, MC 13 (L_1), Panruti local (L_2), Ayakudi local (L_3), Long green (L_4) and Mithi pagal (L_5) and three testers *viz.*, CO1 (T_1), VK 1 Priya (T_2) and Arka Harit (T_3) to study the *per se* performance, combining ability and heterosis. Seeds of the selected parents were sown separately and were selfed for five generations to obtain homozygosity. The selfed seeds were raised in the crossing block and hybridization was attempted in a Line x Tester mating system. Totally 15 hybrids were obtained and evaluated for ten biometrical traits *viz.*, days to first female flowering, days to fruit maturity, number of fruits per vine, fruit length (cm), fruit size index, cavity size index, individual fruit weight (g), yield per vine (g), ascorbic acid content (mg/100g) and TSS (%). The experiment was laid out in a randomized block design and replicated thrice. Observations were taken from randomly selected five plants of each entry. The data were subjected to statistical analysis and are presented in the Tables 1,2,3,4,5a,5b and 5c.

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RESULTS AND DISCUSSION

Performance of the parents can be judged through *per se* performance and general combining ability effects while hybrids can be evaluated based on *per se* performance, specific combining ability effects and heterotic expression. The data on *per se* performance of the parents revealed that the line L_1 (MC 13) recorded the highest *per se* values for fruit length (16.88cm), fruit size index (213.67), cavity size index (63.24), fruit weight, yield per vine and ascorbic acid content. The line L_2 (Panruti local) ranked first for the trait TSS. The highest fruit yield per vine (1286.402) observed by the line L_1 might have acquired through higher values of fruit length and fruit weight. These results are in concomitance with the findings of Richard Kennedy *et al.*, (1996). The line L_5 (Mithi pagal) showed higher *per se* for days to first female flowering (36.66) and days to fruits maturity (10.00). The line L_5 also recorded higher *per se* for number of fruits per vine. The higher number fruits per vine observed by the line L_5 might be due to its earliness. Similar finding also made by Richard Kennedy *et al.*, (1996), among the three testers. T_1 (CO 1) expressed the highest *per se* for individual fruit weight, yield per vine, number of fruits per vine and TSS. The same pollen parent also exhibited the lowest *per se* for days to first female flowering and days to fruit maturity. The tester T_2 ranked high for fruit length, fruit size index and cavity size index. While the tester T_3 expressed good *per se* for ascorbic acid content but poor *per se* for individual fruit weight and yield per vine. Thus the results of the present study indicate the presence of diversity among the parents.

For precise evaluation of parents to test their performance, combining ability analysis is highly helpful (Table 2). The general combining effects (*gca*) of lines revealed that the line L_1 (MC 13) was found to be the best general combiner for most of the important characters like fruit length, fruit size index, cavity size index, yield per vine TSS and ascorbic acid content. It also evidenced that a good combiner for one economic character need not be a good combiner for all the economic characters that contributes the yield. Similar results were also reported by Haripriya (1991) in watermelon. The tester T_3 was found to be a good combiner for days to fruit maturity, fruit length and fruit size index. The diversity in the *gca* effect of various male and female parents can be attributed to genetic as well as geographic diversity present in the parent materials. Higher *gca* effects observed for different characters would be helpful for sorting out the outstanding parents with favourable alleles for different components of yield and quality (Pal *et al.*, 1983). The line L_5 recorded negatively significant *gca* effect for days to first female flower and days to fruit maturity. It was followed by L_3 and T_1. The negative estimates of *gca* for days to first female flower and days to fruit maturity was recorded in the lines L_5, L_3 respectively tester T_1 indicates their effective utilization in the hybridization programme for developing hybrids with earliness.

Mean and *sca* values of hybrids are presented in the Tables 3 & 4. Among the 15 hybrids, three hybrids *viz.*, $L_1 \times T_3$, $L_2 \times T_2$ and $L_1 \times T_1$ were recorded higher values of *per se* for yield per vine, fruit length, cavity size index and individual fruit weight respectively. The cross $L_5 \times T_1$ proved to the best cross combination for number of fruits and days to first female flower. The cross combination indicated that, it was a product of high x low for number of fruits per vine and high x high combination for days to first female flower suggesting the role of additive x dominance and additive x additive type of interactions respectively. The result suggest that the top ranking hybrids have at least one good parent in their cross combination. These findings are in line with the results of Sirohi *et al.*, (1987) in bottle gourd. The results pertaining to the *sca* of hybrids revealed that the two crosses $L_1 \times T_3$ and $L_2 \times T_2$ exhibited higher *sca* for individual furit weight and yield per vine. The parents involved in the cross were a product of good x good combiner for individual fruit weight (Maurya *et al.*, 1993 in bottle gourd). This indicates that the *per se* performance of these hybrids were reflected in their respective *sca* effect. These results are in accordance with the findings of Munshi and Sirohi (1994). The hybrid $L_5 \times T_1$ also showed significant and highly positive *gca* for number of fruits per vine. The hybrid $L_4 \times T_2$ showed higher *sca* effects for furit size index and cavity size index but it was a poor specific combiner for individual fruit

Table 1: Mean performance of parents for biometric traits.

Parents	Days to first Female flowering	Days to fruit maturity	Number of Fruit per vine	Fruit length (cm)	Fruit size index (unit)	Cavity size index	Individual fruit weight (g)	Yield per vine (g)	TSS (%)	Ascorbic acid content (mg/100 g)
L_1	47.99	23.55	14.88	16.88	213.67	63.24	86.66	1286.10	3.583	112.900
L_2	47.44	15.00	13.33	12.88	146.66	50.12	56.10	776.66	4.340	91.933
L_3	38.11	10.55	24.33	5.42	58.01	20.83	17.22	416.66	2.700	88.299
L_4	50.77	16.66	13.88	13.77	121.22	44.53	45.55	641.66	2.200	93.000
L_5	36.66	10.00	36.44	5.01	53.07	14.89	11.55	431.99	1.960	87.00
T_1	46.22	15.99	18.77	17.99	161.65	59.66	78.88	1438.33	4.476	100.300
T_2	46.33	16.11	13.33	19.00	249.05	68.94	74.44	952.99	2.833	112.166
T_3	52.99	16.88	14.77	14.33	179.33	45.33	58.88	912.22	1.836	121.466
SEd L	1.40	0.72	0.99	0.51	7.96	2.16	4.00	76.17	0.173	4.457
SEd T	0.19	0.10	0.13	0.07	1.12	0.36	0.56	10.77	0.024	0.630

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Table 2 : General Combining ability effects (*gca*) of parents for biometric traits.

Parents	Days to first Female flowering	Days to fruit maturity	Number of Fruit per vine	Fruit length (cm)	Fruit size index	Cavity size index	Individual fruit weight (g)	Yield per vine (g)	TSS (%)	Ascorbic acid content (mg/100 g)
L_1	0.15	2.40	-3.53	4.20	74.19	19.85	21.97	327.77	0.960	11.888
L_2	1.22	2.22	-3.97	1.43	24.83	8.54	21.97	269.73	0.555	2.188
L_3	-1.22	-2.99	4.18	-4.39	-51.19	-18.42	-26.51	-331.01	-0.386	-0.322
L_4	3.45	1.51	-5.01	2.57	26.37	15.67	11.64	61.03	-0.489	-3.522
L_5	-3.59	-3.45	8.33	-3.81	-74.19	-25.64	-29.07	-327.53	-0.639	-10.233
T_1	-1.95	-0.19	1.76	-0.24	-8.43	-0.48	0.29	48.09	0.432	-2.402
T_2	2.65	-0.37	-0.81	-0.29	10.01	-1.03	-1.76	-50.79	-0.233	0.237
T_3	-0.70	0.56	-0.95	0.53	8.42	1.51	1.45	1.39	-0.199	2.164
CD 5% (L)	0.662	0.341	0.450	0.204	3.750	1.232	1.88	35.91	0.082	2.100
CD 5% (T)	0.513	0.264	0.360	0.189	1.126	0.369	0.566	10.772	0.063	1.620

Table 3 : *Per se* performance of Hybrids.

Hybrids	Days to first female flower	Days to fruit maturity	Number of Fruit per vine	Fruit length	Fruit size index	Cavity size index	Individual fruit weight	Yield per vine	TSS (%)	Ascorbic acid (mg/100 g)
$L_1 \times T_1$	49.44	18.11	15.66	19.22	257.44	66.88	71.11	1125.55	4.366	101.333
$L_1 \times T_2$	50.33	15.55	14.99	15.55	194.88	59.33	48.77	756.89	3.183	113.333
$L_1 \times T_3$	45.33	16.33	17.99	19.77	273.49	79.09	87.22	1573.89	4.100	111.833
$L_2 \times T_1$	44.44	15.99	16.66	14.55	176.16	58.79	61.11	1014.99	4.436	93.000
$L_2 \times T_2$	57.44	17.11	17.44	15.66	195.37	51.90	82.21	1418.33	3.033	100.000
$L_2 \times T_3$	46.89	16.33	13.22	15.99	206.15	60.68	63.78	848.88	2.966	105.033
$L_3 \times T_1$	45.33	9.89	25.11	6.22	67.11	20.68	24.44	611.11	3.176	91.799
$L_3 \times T_2$	46.98	9.99	22.99	10.88	128.51	35.08	18.33	426.11	2.166	100.000
$L_3 \times T_3$	49.11	13.89	23.66	11.66	153.99	34.72	18.89	442.78	2.266	98.700
$L_4 \times T_1$	50.99	15.89	15.32	15.66	197.55	63.41	61.11	950.55	2.833	97.000
$L_4 \times T_2$	53.89	16.11	14.11	18.11	230.44	73.33	57.77	817.77	1.900	87.235
$L_4 \times T_3$	50.55	16.22	14.78	15.88	154.33	55.99	57.22	887.77	2.566	96.666
$L_5 \times T_1$	41.88	10.44	34.78	13.05	98.27	30.72	18.99	663.99	1.966	90.000
$L_5 \times T_2$	46.44	10.66	25.11	8.22	89.55	18.11	19.44	451.11	3.160	86.400
$L_5 \times T_3$	45.99	11.33	24.33	9.22	92.77	19.98	15.55	375.33	1.710	84.366
SE(L)	1.406	0.723	0.988	0.519	7.960	2.610	4.508	76.175	0.175	4.457
SE(T)	0.198	0.102	0.139	0.078	1.126	0.360	0.566	10.772	0.024	0.630

weight and yield per vine. This may be due to the changes in yield which was not components need not result in changes in yield. Such findings are in agreement with the reports of Haripriya (1991) in watermelon.

The heterotic expression of 15 F_1 hybrids was presented in the Table 5a, 5b, and 5c revealed that profound heterosis for different characters. Among the three hetorosis, the standard hetorosis (diii) has to be considered for selecting the best hybrids for commercial exploitation. In the present experiment for all the ten characters studied MC 13 was used as a standard check. Among the 15 F_1 hybrids evaluated $L_1 \times T_3$ and $L_2 \times T_2$ expressed the greatest heterotic effect over check variety for individual fruit weight and yield per vine. The involvement of atleat one good parent in the heterotic combination suggests a complementary type of heterosis wherein one parent compensates for the dificiency of the other. This could possible due to additive x dominant type of epistatic interaction. The cross $L_1 \times T_3$ registered the highest heterosis over check parent for fruit length, furit size index and cavity size index. The high heterosis observed in this cross for above characters may be due to the higher combination of parents L_1 and T_3 involved in fruit length and fruit size index indicates that good x good combiner resulted in the best hybrid (Srivastava and Nath, 1983).

Bitter gourd being monoecious crop earliness is a desirable character. The crosses $L_1 \times T_3$ and $L_3 \times T_2$ had recorded good heterotic expression for days to fruit maturity over check parent. Heterosis for earliness in bitter gourd was

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Table 4: Specific combining ability effects (*sca*).

Hybrids	Days to first female flower	Days to fruit maturity	Number of Fruit per vine	Fruit length	Fruit size index	Cavity size index	Individual fruit weight	Yield per vine	TSS (%)	Ascorbic acid (mg/100 g)
$L_1 \times T_1$	2.87*	1.64*	-2.32*	1.27*	23.94*	-1.07	1.77	75.45*	0.050	-4.675*
$L_1 \times T_2$	-0.83	-0.74*	-0.41	-2.34*	-47.10*	-8.07*	-18.50*	- 344.92*	-0.466*	4.051*
$L_1 \times T_3$	-2.04*	-0.89*	2.73*	1.06*	23.16*	9.14*	16.72*	420.38*	0.416*	0.624
$L_2 \times T_1$	-3.20*	-.29*	-0.88	-0.67*	-7.98*	2.15*	-8.21*	- 127.97*	0.524*	-3.942*
$L_2 \times T_2$	5.20*	0.99*	2.48*	0.55*	2.80	-4.19*	14.94*	374.55*	-0.212	-0.417*
$L_2 \times T_3$	-2.00*	-0.71*	-1.61*	0.06	5.17	2.04	-6.72*	- 246.57*	-0.312*	3.524
$L_3 \times T_1$	0.14	-1.81*	-0.56	-3.13*	-41.00*	-8.99*	3.59*	68.87*	0.207*	-2.631
$L_3 \times T_2$	-2.31*	-0.88*	-0.11	1.59*	11.96*	5.95*	-0.46	-16.92	-0.136	2.928
$L_3 \times T_3$	2.67*	2.07*	0.69	1.55*	29.04*	3.05*	-3.13	-51.94	-0.070	-0.297
$L_4 \times T_1$	1.13*	0.01	-1.18*	-0.65*	11.87*	-0.35	2.12	16.28	-0.032	5.768*
$L_4 \times T_2$	-0.56	0.41	0.19	1.85*	36.32*	10.11*	0.83	-17.29	-0.300*	-6.637*
$L_4 \times T_3$	-0.56	-0.41	0.99*	-1.19*	-48.19*	-9.76*	-2.95	1.01	0.333*	0.868
$L_5 \times T_1$	-0.94	-0.18	4.94*	-3.13*	13.17*	8.26*	0.71	18.28*	-0.749*	5.480*
$L_5 \times T_2$	-0.98	0.22	-2.15*	-1.65*	-3.99	-3.79*	3.20	4.59	1.116	-0.759
$L_5 \times T_3$	1.03*	-0.04	-2.79*	-1.47*	-9.18*	-4.47*	-3.91*	- 128.87*	-0.366*	-4.719*
CD (5%)	1.105	0.591	0.807	0.424	6.501	2.134	3.272	62.200	0.142	3.650

*Significant at 5%

Table 5a: Heterosis for different characters in bitter gourd.

Hybrids	Days to first Female flower			Days to fruit maturity			Number of fruits per vine		
	di	dii	diii	di	dii	diii	di	dii	diii
$L_1 \times T_1$	4.95	3.01	2.01	-8.42*	-23.11**	-23.11**	-6.93	-16.57**	5.26
$L_1 \times T_2$	6.71*	4.86	4.86	-4.57	-33.97**	-33.97**	6.30	0.74	0.75
$L_1 \times T_3$	-9.35**	-13.62**	-4.63	-19.24**	-30.67**	-30.67**	21.34**	20.89**	20.89**
$L_2 \times T_1$	-5.11	-6.33	-7.41*	3.22	0.00	-32.08**	3.79	-11.26	11.91
$L_2 \times T_2$	22.57**	21.08**	19.68**	9.97*	9.17	-27.37**	30.86**	30.86**	17.17**
$L_2 \times T_3$	-6.63*	-11.53**	-2.13	2.43	-3.29	-30.67**	-5.95	-10.56	-7.22
$L_3 \times T_1$	7.50*	-1.92	-2.13	-25.52**	-38.19**	-58.02**	16.51**	3.21	68.67**
$L_3 \times T_2$	11.27**	1.39	-5.55	-25.52**	-37.95**	-57.56**	22.13**	-5.48	54.48**
$L_3 \times T_3$	7.80	-7.33	-2.13	1.21	-17.77**	-41.04**	21.02**	-2.74	58.96**
$L_4 \times T_1$	5.16	0.44	6.25	-2.71	-4.66	-32.55**	-6.18	-18.39**	2.91
$L_4 \times T_2$	10.98**	6.13	12.27**	-1.71	-3.34	-31.62**	3.66	1.58	-5.29
$L_4 \times T_3$	-2.59	-4.61	5.32	-3.31	-3.95	-31.14**	3.14	0.00	-7.39
$L_5 \times T_1$	1.06	-9.38*	12.73**	-19.68**	-34.75**	-55.68**	25.96**	-4.57	13.36**
$L_5 \times T_2$	11.91**	0.24	-3.24	-18.32**	-33.81**	-54.73**	3.84	-31.33**	68.65**
$L_5 \times T_3$	2.60	-13.20**	-4.17	-15.72**	-32.91**	-51.89**	-4.99	33.24**	63.43**

**Significant at 1% level, *Significant at 5% level

also reported by Ranpise *et al.*, (1992). It was note worth that the parents which expressed comparatively high mean performance have not always resulted in heterotic combinations excepting few. This may be due to the mutual cancellation of gene effects or the involvement of large number of interallelic interactions esecially of duplicate type of epistasis either through additive x dominant or dominant x dominant.

From the study it was inferred that the two hybrids $L_1 \times T_3$ and $L_2 \times T_2$, which registered standard heterosis of 22.38 per cent and 10.28 per cent over check variety can be recommended for commercial exploitation. But these hybrids need to be further test verified for yield, stability and genotype x environment.

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Table 5b: Heterosis for different characters in bitter gourd.

Hybrids	Fruit length			Fruit size index			Cavity size index		
	di	dii	diii	di	dii	diii	di	dii	diii
$L_1 \times T_1$	10.20**	6.80*	13.82**	37.18**	20.48**	20.48**	8.83*	5.75	5.75
$L_1 \times T_2$	-13.32**	-18.14**	-7.80*	-15.79**	-21.77**	-8.82	-10.23*	-13.94	-6.18
$L_1 \times T_3$	26.68**	17.09**	17.09**	39.18**	27.99**	27.99**	45.68**	25.05**	25.05**
$L_2 \times T_1$	-5.75	-19.13**	13.82**	14.27**	8.97**	-17.55**	7.11	-1.45	-7.03
$L_2 \times T_2$	-1.76	-17.56**	-7.24*	-1.25	-21.55**	-8.56	-12.82**	-24.72**	-17.94**
$L_2 \times T_3$	17.55**	11.63**	-5.27	26.47**	14.96**	-3.52	27.14**	21.06**	-4.06
$L_3 \times T_1$	-46.88**	-65.44**	-63.17**	-38.90**	-58.49**	-68.59**	-48.61**	-65.33**	69.30**
$L_3 \times T_2$	-10.86*	-42.72**	-35.55**	-16.30**	-14.13**	-27.93**	4.96	-23.41**	-45.10**
$L_3 \times T_3$	18.10**	-18.61**	-30.93**	29.77**	-14.13**	-27.93**	4.96	-23.41**	-45.10**
$L_4 \times T_1$	-1.41	-12.97**	-7.24*	39.67**	22.21**	-7.55	21.72**	6.29	6.27
$L_4 \times T_2$	10.51**	-4.68	7.24*	24.47**	-7.47	7.85	29.24**	6.36	15.95**
$L_4 \times T_3$	13.05**	10.86**	-5.92	2.70	-13.94**	-27.77**	24.63**	23.53**	-11.46**
$L_5 \times T_1$	13.44**	-27.47**	-22.70**	-8.47	-39.21**	-54.01**	-17.59**	-48.51**	-51.43**
$L_5 \times T_2$	-31.55**	-56.74**	-51.32**	-40.72**	-64.04**	-58.09**	-56.80**	-73.73**	-71.36**
$L_5 \times T_3$	-4.69	-35.65	-75.04**	-20.16**	-48.27**	-56.58**	-33.62**	-55.91**	-68.40**

**Significant at 1% level, *Significant at 5% level

Table 5c: Heterosis for different characters in bitter gourd.

Hybrids	Single fruit weight			Yield per vine			TSS			Assorbic acid		
	di	dii	diii	di	dii	diii	di	dii	diii	di	dii	diii
$L_1 \times T_1$	-14.09**	-14.09**	-14.09**	-17.37**	-21.75**	-21.75**	-12.48	8.854	-2.457	21.860**	-4.346	-9.684*
$L_1 \times T_2$	-39.45**	-39.45**	-39.45**	-32.39**	-41.15**	-41.18**	-7.792	-11.162	-11.162	0.710	0.383	0.383
$L_1 \times T_3$	19.85**	19.85**	19.85**	43.19**	22.38**	22.36**	51.291**	14.418*	14.418*	-4.565	-7.930	-0.944
$L_2 \times T_1$	-9.47	-9.47	-9.47	-8.35	-29.43**	-21.08**	0.642	-0.893	23.813**	-3.242	-7.278	-17.626**
$L_2 \times T_2$	25.94**	25.94**	25.94**	64.00**	48.82**	10.28	-3.989	-31.643**	-17.209**	-2.008	-10.846*	-11.426*
$L_2 \times T_3$	10.92	10.92	10.92	0.52	-6.94	-33.99**	-3.989	-31.643**	-17.209**	-1.562	-13.529*	-6.967
$L_3 \times T_1$	-49.13**	-49.13**	-49.13**	-34.11**	-57.51**	-52.48**	-11.472*	-29.039**	-11348*	-2.651	-8.474	-18.689**
$L_3 \times T_2$	-59.99**	-59.99**	-59.99**	-37.78**	-55.29**	-52.48**	-21.686**	-23.529**	-39.534**	-0.232	-10.846*	-11.426*
$L_3 \times T_3$	-50.37**	-50.37**	-50.37**	-33.36**	-51.46**	65.57**	-0.073	-16.049*	-36.744**	-5.895	-18.743**	-12.577**
$L_4 \times T_1$	-1.79	-1.79	-1.79	-8.60	-33.91**	-26.09**	-15.127**	-36.708**	-20.930**	0.362	-3.290	-14.083**
$L_4 \times T_2$	-3.70	-3.70	-3.70	2.56	-14.19	-36.41**	-24.503	-32.941**	-46.976**	-14.963**	-22.228**	-22.733**
$L_4 \times T_3$	9.57	9.57	9.57	14.27	-2.88	-30.97**	27.167**	16.66**	-28.372**	-9.853*	-20.417**	-14.378**
$L_5 \times T_1$	-57.99**	-57.99**	-57.99**	–28.99**	-53.84**	-48.37**	-38.891**	-56.068**	-45.116**	-3.897	-10.269	-20.283*
$L_5 \times T_2$	-52.78**	-54.78**	-54.78**	-34.86**	-52.66**	-64.92**	32.127**	11.760	-11.620*	-13.238**	-22.971**	-23.472**
$L_5 \times T_3$	-55.84**	-55.84**	-55.84**	-44.16**	-58.86**	-70.82**	-9.569	-12.414	-52.093**	-19.059**	-30.543**	-25.273**

**Significant at 1% level, *Significant at 5% level

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Effectiveness and Efficiency of Gamma Rays in Pigeonpea (*Cajanus cajan* (L.) Millsp.)

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The present investigation was undertaken with an objective of inducing genetic variability in Pigeonpea [*Cajanus cajan* (L.) Millsp.], employing technique, mutation breeding. It was also intended to study the effect of different doses of Gamma rays on the frequency of chlorophyll mutations in M_2 generation. Biological damage in M_1 generation of Pigeonpea, the mutagenic effectiveness and efficiency were assessed. On the basis of present findings, it is suggested that 200Gy dose of Gamma rays is the preferable and effective dose for induction of desirable mutations in Pigeonpea.

Keywords : *Cajanus cajan*, Gamma rays, Efficiency.

INTRODUCTION

Cajanus cajan (L.) Millsp, commonly known as Pigeonpea, Tur or Red gram is a prime pulse crop of Indian sub continent, and good source of protein, minerals and vitamins. Pigeonpea, being a self pollinated crop, the available genetic variability has been almost exploited for improvement by conventional breeding methods. Induced mutagenesis has been used very widely in crop plants to create genetic variability in traits of economic value (Satpute, 2009). Therefore it becomes necessary to create genetic variability through induced mutations. Chlorophyll mutations offer one of the most reliable indices for the assessment of genetic effects of mutagenic treatment. According to Miller (1968), in spite of impaired seed production the chlorophyll mutants are potentially useful in understanding of different physiological functions, various biochemical reactions and pathological invasion. Although mutagens bring about changes in nucleotide sequence of DNA the mode of action of each mutagen is distinct. More ever, a mutagen may effectively bring about mutations, but the accompanying undesirable effects like lethality or sterility may decrease its efficiency. Thus, in order to exploit induced mutagenesis for crop improvement, the basic studies on effectiveness and efficiency of a mutagen in a crop are necessary (Badere and Chaudhary, 2007).

In present investigation efforts were made to different doses of Gamma rays on Pigeonpea (ICPL-87) in terms of chlorophyll mutations to find out effective dose, which induced desirable mutations.

MATERIAL AND METHODS

Authentic germplasms of Pigeonpea var., ICPL-87 were, procured from 'Pulses improvement Division' of Mahatma Phule Agricultural University, Rahuri (Ahmednagar district, Maharashtra). The seeds were irradiated with different doses Gamma rays (100, 200, 300 and 400Gy) from Institute of Science, Aurangabad, The mutagen treated and control (untreated) seeds were sown in the field in (RDB) Randomized block design with three replications in *Kharif* 2007. The seeds of all individual M_1 plants were harvested separately and they were sown in the field during next *Kharif* 2008 to rise to rise M_2 generation. Mutagenic effect was recorded by using the parameters like seed germination, plant survival, pollen sterility and chlorophyll mutants. The treated as well as control seeds after germination were carefully screened for chlorophyll mutation in M_2 generation. Based on this data the mutagenic effectiveness and efficiency were calculated by using the formulae suggested by Konzak *et al.*, (1965).

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (MF)}}{\text{Dose}} = \text{MF/Gy}$$

Where,

MF = % of chlorophyll mutations in M_2 generation.

Gy = Grays, unit of gamma radiation.

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency (MF)}}{\text{Biological damage}} = \text{MF/L, MF/I, MF/S}$$

Where,

L = % of lethality in M_1 generation.

I = % of seedling injury in M_1 generation.

S = % of pollen sterility in M_1 generation.

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Table 1: Effectiveness and efficiency of Gamma rays in Pigeonpea in M_2.

Dose	Frequency of Chlorophyll Mutants (%) Chlorophyll Mutants (%)	Effectiveness MF/dose MF/dose	Lethality (L) % (L) %	Efficiency (MF/L) (MF/L)
100Gy	5.45	0.055	9.86	0.55
200Gy	15.18	0.076	11.6	1.31
300Gy	5.14	0.017	13.82	0.37
400Gy	3.46	0.009	23.48	0.15

SE ± 0.034, CV % . 0.98, CD 5% : 0.10, CD 1% : 0.14

Table 2: Effect of Gamma rays on seed germination, plant survival and seed sterility in Pigeonpea in M_1 generation.

Dose	Seed germination (%)	SE	Plant Survival (%)	SE	Pollen sterility (%)	SE
100Gy	84	± 0.19	90.14	± 0.14	4.61	± 0.16
200Gy	76	± 0.11	88.40	± 0.15	7.14	± 0.21
300Gy	68	± 0.22	86.18	± 0.14	9.04	± 0.21
400Gy	49	± 0.19	76.52	± 0.19	30.70	± 0.31

RESULTS AND DISCUSSION

The M_2 population of pigeonpea shows different chlorophyll mutants like *Chlorina, Xantha, Albina* and *Striata*. The frequency of chlorophyll mutants showed an increase at lower dose, but gets decreased at higher dose of gamma rays. In the present investigations, the higher frequency of chlorophyll mutant is recorded in 200Gy dose, while lower frequency is rocorded in 400Gy dose of Gamma rays (Table 1).

Mutagenic Effectiveness : The results (Table 1) have shown tnat, effectiveness of mutagenic treatments differed considerably. The mutagenic effectiveness showed a trend, which was inversely proportional to the increasing dose (execpt 100Gy) of mutagens in pigeonpea. The mutagenic effectiveness varied from 0.009 to 0.076 in Gamma rays. The present data further revealed that the lower dose of Gamma rays (200Gy) was more effective in causing chlorophyll mutation. From the data it is concluded that the 200Gy dose is more effective while the 400Gy dose is less effective in inducing chlorophyll mutation in Pigeonpea ICPL-87. The decrease in effectiveness with increasing dose of mutagen has been reported by Khan *et al.*, (1973), Sinha and Akhaury (1969), Nadarajan and Ramalingam, (1982), Sundaram, (1974), Rao *et al.*, (1983) in pigeonpea.

Mutagenic Efficiency : Mutagenic efficiency is the ratio of frequency of chlorophyll mutations induced in M_2 generation to various biological damages (such as plant survival, pollen sterility, seed germination and lethality) observed in M_1 generation. Experimental results obtained on mutagenic efficiency of the mutagens used in the present investigation are presented in Table 1. Mutagenic efficiency varied from 0.15 to 1.31. It varied with respect with different parameters. The mutagenic efficiency linearly increased at the 100Gy 200Gy, while decreased from 200Gy to 300Gy and 400Gy dose of Gamma rays (Table 1).

The data regarding pollen sterility, plant survival and seed germination in relation to efficiency have shown a reducing trend with the gradual increase in concentration of Gamma rays in Pigeonpes ICPL-87 (Table 2). The mutagenic treatment of Gamma rays caused considerable reduction in seed germination, pollen Sterility and plant survival. Similar observation has been recorded by Biradar (2004), Shinde (2007) in Pigeonpea, Satpute (2009) in lentil. The result of present study indicated that lower dose (200Gy) of Gamma rays is more effective for the induction of desirable mutations for Pigeonpea improvement.

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Evaluation of Genetic Divergence in Different Varieties of *Zea mays* L.

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Analysis of genetic divergence using Mahalanobis's D^2 statistic was carried out in eleven varieties of *Zea mays* L. These varieties were grouped into four clusters. The maximum intercluster D^2 value was obtained between cluster III and IV and I and IV. Therefore, it is suggested that for developing better variety genotype of cluster IV could be utilized in hybridization programme with the genotype of cluster III and I respectively.

Keywords : Genetic Divergence, *Zea mays* L., D^2 statistic.

INTRODUCTION

Genetic diversity in a trait is essential for crop improvement programme. Selection of diverge parents belonging to distant groups leads to a wide spectrum of gene recombination for quantitatively inherited traits (Jain, 1981). Mahalanobis's D^2 statistic (1936, 1949) has been found to be a powerful tool to estimate genetic divergence among population. Kumar (2008) analysed genetic divergence in Maize on the basis of phenotypic traits *viz.* Plant height, grain weight, grain yield *etc.* In the present study the D^2 analysis has been done to study the role of Chromatin length of Chromosomes to find out the genotypic closeness among different varienties of *Zea mays* L..

MATERIAL AND METHODS

Eleven varieties of Zea mays L. *viz.* Pop corn, KH-9451, Shanker, K-51, DMH-849, Baby corn, K-99, Proagro-4212, Super Gold-651, Ganga safed-2 and Sweet cron were taken for the analysis of genetic divergence. Root tips from germinating seed of all the selected varieties were excised and pretreated in para-dichloro benzene, fixed in 1:3 aceto-alcohol for 24 hours and then the roots were transferred to 70% alcohol for preservation. For staining, the root tips were hydrolysed in 1N HCl at 60°C for 15 minutes and then stained in 2% aceto-carmine solution. Slides were prepared by squash technique. Ten well separated chromosome plates were taken for experimental purpose. The genetic divergence was assessed using Mahalanobis's D^2 statistic (1936, 1949).

Following data were recorded for D^2 analysis-

a) The absolute length of chromosome.
b) The relative length of each chromosome.
c) The angular value of the chromosome.
d) The log value: the ratio of long arm and short arm was converted into logarithmic scale.

RESULTS AND DISCUSSION

Mitotic studies carried out in the present investigation showed a uniformity in the diploid chromosome complement *i.e.*, 2n = 20 in all the varieties of *Zea mays* L.. The analysis of genetic divergence in the different varieties of *Zea mays* under investigation indicated thatl all the varieties could be grouped into four constellations (Fig. 1 and Table 1-3). The constellation CI consisted of three varieties V_2, V_3 and V_6 represents KH-9451, Shanker and Baby corn. The constellation CII consists of V_5, V_{10} and V_{11} which were respectively for DMH-849, Ganga safed-2 and Sweet corn. The constellation CIII consisted of three varieties V_7, V_8 and V_9 namely K-99, Proagro-4212 and Super Gold-651 whereas, the constellation CIV consisted of two varieties V_1 and V_4 which represents pop corn and K-51 respectively. The analysis of genetic divergence indicated that intraconstellar distance in the constellation CI, CII, CIII and CIV were 32.43, 66.07, 360.51 and 34.12. The interconstellar distance between CI, CII,CI,CIII,CI-CIV,CII-CIII, CII-CIV, CIII-CIV were respectively 687.46, 2205.34, 3451.98, 2468.26, 3350.95 and 4490.12.The highest and lowest D^2 value in the intraconstellar divergence were observed in CII and CI respectively whereas the highest and lowest D^2 value in the interconstellar divergence were observed between CIII-CIV and CI-CII. The maximum intercluster D^2 value was obtained between CIII-IV and CI-CIV. Therefore, it is suggested that for developing better variety genotype of CIV could be utilized in hybridization programme with the genotype of CIII and CI respectively (Chaturvedi and Maurya, 2005). The clustering pattern obtained in the present investigation revealed that varieties belonging to the same species might fall in different constellations. Such grouping of varieties in different constellations might be the outcome of variation in genotype. The higher D^2 values within a constellation were indication of high degree of divergence in the chromosome make-up. Thus, the varieties falling in the same cluster were genotypically more allied than those of in different constellation. Similar results were reported earlier which support present findings Mahalanobis (1936, 1949), Kumar and Akhoury (1996), Chaturvedi and Maurya (2005), Kumar and Mukherjee (2005),

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Table 1 : The relative length (in μ) of individual chromosome and its conversion into angular value (a) and the ratio of long arm/short arm and its conversion into logarithmic scale (b).

Varieties under consideration	Chromosome I	Chromosome II	Chromosome III	Chromosome IV	Chromosome V	Chromosome VI	Chromosome VII	Chromosome VIII	Chromosome IX	Chromosome X
Zea mays var. Pop corn	a. 14.87 ± 0.22 (22.63)	a. 14.71 ± 0.12 (22.55)	a. 13.76 ± 0.20 (21.72)	a. 10.75 ± 0.72 (19.09)	a. 11.55 ± 0.41 (19.82)	a. 9.33 ± 0.46 (17.76)	a. 8.54 ± 0.44 (16.95)	a. 7.59 ± 0.27 (15.80)	a. 5.22 ± 0.22 (13.18)	a. 3.63 ± 0.07 (10.94)
	b. 1.65 ± 0.14 (0.21)	b. 1.1 ± 0.04 (0.04)	b. 1.17 ± 0.04 (0.06)	b. 1.17 ± 0.41 (0.23)	b. 1.23 ± 0.10 (0.08)	b. 1.96 ± 0.51 (0.29)	b. 2.36 ± 0.67 (0.37)	b. 1.28 ± 0.18 (0.10)	b. 1.62 ± 0.22 (0.20)	b. 1.70 ± 0.34 (0.23)
Zea mays var. KH-9451	a. 17.88 ± 0.41 (24.95)	a. 11.47 ± 0.12 (19.73)	a. 12.14 ± 0.26 (20.36)	a. 13.68 ± 0.27 (21.64)	a. 13.68 ± 0.18 (21.64)	a. 9.71 ± 0.22 18.15	a. 8.16 ± 0.22 (16.54)	a. 5.96 ± 0.12 (14.06)	a. 3.97 ± 0.07 (11.39)	a. 3.31 ± 0.09 (10.47)
	b. 1.48 ± 0.29 (0.17)	b. 6.2 ± 1.19 (0.79)	b. 2.63 ± 0.43 (0.42)	b. 1.50 ± 0.20 (0.24)	b. 1.87 ± 0.38 (0.18)	b. 2.7 ± 0.66 (0.27)	b. 3.1 ± 0.84 (0.43)	b. 1.4 ± 0.24 (0.49)	b. 1.4 ± 0.24 (0.14)	b. 1.6 ± 0.20 (0.20)
Zea mays var. Shanker,	a. 23.80 ± 0.85 (29.20)	a. 16.90 ± 0.20 (24.27)	a. 13.57 ± 0.21 (21.56)	a. 11.66 ± 0.56 (19.91)	a. 9.28 ± 0.45 (17.66)	a. 7.38 ± 0.44 (15.68)	a. 6.19 ± 0.30 (14.56)	a. 4.75 ± 0.16 (12.52)	a.3.57 ± 0.16 (10.78)	a. 2.85 ± 0.12 (9.63)
	b. 1.56 ± 0.19 (0.19)	b. 2.91 ± 0.96 (0.46)	b. 5.1 ± 0.75 (0.70)	b. 3.9 ± 1.10 (0.59)	b. 2.31 ± 0.51 (0.36)	b. 2.37 ± 0.50 (0.17)	b. 1.97 ± 0.29 (0.29)	b. 2.±0.32 (0.30)	b. 1.5 ± 0.22 (0.18)	b. 1.4 ± 0.24 (0.15)
Zea mays var. K-51	a. 15.02 ± 0.84 (22.79)	a. 14.63 ± 0.34 (22.46)	a. 13.51 ± 0.23 (21.56)	a. 13.73 ± 0.18 (21.72)	a. 11.26 ± 0.30 (19.55)	a. 9.68 ±0.36 (18.05)	a. 8.33 ± 0.33 (16.74)	a. 6.30 ± 0.20 (14.54)	a. 50 ± 0.26 (12.25)	a. 2.92 ± 0.18 (9.81)
	b. 2.72 ± 0.69 (0.43)	b. 3.15 ± 0.96 (0.49)	b. 2.86 ± 0.75 (0.45)	b. 1.65 ± 0.28 (0.21)	b. 2.67 ± 0.94 (0.42)	b. 2.32 ± 0.70 (0.36)	b. 1.54 ± 0.26 (0.18)	b. 1.53 ± 0.16 (0.18)	b. 1.8 ± 0.25 (0.25)	b. 1.1 ± 0.10 (0.04)
Zea mays var. DMH-849	a. 18.06 ± 0.47 (25.10)	a. 15.33 ± 0.43 (23.03)	a. 13.89 ± 0.34 (21.72)	a. 13.18 ± 0.22 (21.23)	a.9.52 ± 0.35 (17.95)	a. 8.54 ± 0.33 (16.95)	a.7.40 ± 0.43 (15.79)	a. 6.10 ± 0.25 (14.36)	a. 4.64 ± 0.18 (12.39)	a. 3.42 ± 0.11 (10.63)
	b. 2.92 ± 0.89 (0.47)	b. 4.12 ± 1.37 (0.61)	b. 2.89 ± 0.68 (0.46)	b. 1.36 ± 0.07 (0.13)	b. 2.24 ± 0.74 (0.35)	b. 2.45 ± 0.73 (0.39)	b. 1.80 ± 0.13 (0.26)	b. 1.87 ± 0.13 (0.27)	b. 1.4 ± 0.19 (0.15)	b. 1.4 ± 0.25 (0.15)
Zea mays var. Baby corn	a. 16.07 ± 0.15 (23.58)	a. 13.95 ± 0.44 (21.89)	a.13.23 ± 0.24 (21.30)	a. 10.63 ± 0.32 (19.20)	a. 10.63 ± 0.13 (19.00)	a.9.22 ± 0.22 (19.66)	a. 7.80 ± 0.12 (16.22)	a.7.56 ± 0.20 (15.80)	a. 6.14 ± 0.06 (14.86)	a. 4.73 ± 0.13 (12.52)
	b. 2.93 ± 0.58 (0.47)	b. 3.87 ± 1.17 (0.59)	b. 2.86 ± 0.72 (0.46)	b. 3 ± 0.83 (0.48)	b. 2.65 ± 1.15 (0.42)	b. 2.3 ± 0.69 (0.36)	b. 2.66 ± 0.64 (0.42)	b. 1.65 ± 0.15 (0.22)	b. 1.6 ± 0.10 (0.20)	b. 1.4 ± 0.19 (0.15)
Zea mays var. K-99	a. 19.67 ± 0.24 (26.28)	a. 16.34 ± 0.28 (23.81)	a. 13.02 ± 0.20 (21.13)	a. 11.36 ± 0.24 (19.64)	a.11.36 ± 0.24 (19.64)	a. 8.86 ± 0.22 (17.26)	a. 7.20 ± 0.18 (15.56)	a.5.54 ± 0.13 (13.56)	a.3.88 ± 0.06 (11.24)	a.1.39 ± 0 (6.56)
	b. 1.19 ± 0.17 (0.28)	b. 2.57 ± 0.42 (0.41)	b. 3.58 ± 0.90 (0.55)	b.2.93 ± 0.68 (0.46)	b. 1.37 ± 0.17 (0.14)	b. 1.32 ± 0.09 (0.12)	b. 1.57 ± 0.37 (0.20)	b. 1.6 ± 0.19 (0.20)	b. 1.8 ± 0.45 (0.25)	b.1 ± 0 (0)
Zea mays var. Proagro-4212	a. 17.49 ± 0.32 (24.65)	a.13.66 ± 0.31 (21.64)	a. 12.78 ± 0.15 (20.88)	a. 12.55 ± 0.15 (20.70)	a.10.54 ± 0.15 (18.91)	a.8.52 ± 0.24 (24.95)	a. 8.52 ± 0.07 (16.95)	a.6.65 ± 0.11 (15.23)	a. 5.16 ± 0.12 (13.05)	a.3.81 ± 0.07 (11.24)
	b. 1.66 ± 0.23 (0.22)	b. 2.13 ± 0.51 (0.33)	b. 1.49 ± 0.25 (0.17)	b. 1.25 ± 0.06 (0.09)	b. 1.54 ± 0.37 (0.19)	b. 2.95 ± 0.879 (0.47)	b. 1.13 ± 0.08 (0.05)	b. 1.63 ± 0.15 (0.21)	b. 1.6 ± 0.10 (0.20)	b. 1.6 ± 0.25 (0.20)
Zea mays var. Super Gold-651	a. 20.51 ± 0.42 (26.92)	a. 16.92 ± 0.39 (24.27)	a.13.33 ± 0.45 (21.39)	a. 12.30 ± 0.22 (2.53)	a. 10.25 ± 0.19 (18.63)	a.7.18 ± 0.12 (15.45)	a. 6.15 ± 0.06 (14.36)	a. 5.38 ± 0.11 (13.31)	a.4.36 ± 0.12 (11.97)	a.3.59 ± 0.06 (10.78)
	b. 3.44 ± 1.90 (0.54)	b. 2.11 ± 0.52 (0.32)	b. 5.13 ± 1.63 (0.71)	b. 1.19 ± 0.054 (0.075)	b.1.48 ± 0.26 (0.17)	b.2.1 ± 0.53 0.32	b..9 ± 0.54 (0.28)	b. 2.2 ± 0.34 (0.34)	b. 1.2 ± 0.20 (0.07)	b. 1.8 ± 0.20 (0.26)
Zea mays var. Ganga Safed-2	a. 16.29 ± 0.48 (23.73)	a.13.64 ± 0.57 (21.64)	a. 14.46 ± 0.72 (22.30)	a.11.40 ± 0.53 (19.73)	a. 11.20 ± 0.60 (19.55)	a.9.37 ± 0.18 (17.76)	a. 10.18 ± 0.49 (18.53)	a. 5.91 ± 0.11 (14.06)	a. 4.68 ± 0.15 (12.39)	a.2.85 ± 0.18 (9.63)
	b. 2.37 ± 0.86 (0.37)	b. 3.73 ± 1.90 (0.57)	b. 1.65 ± 0.38 (0.22)	b. 1.84 ± 0.32 (0.20)	b. 2.12 ± 0.74 (0.33)	b. 3.35 ± 1.56 (0.17)	b.2.06 ± 0.52 (0.31)	b. 2.5 ± 0.65 (0.39)	b.1.6 ± 0.19 (0.20)	b. 1.3 ± 0.06 (0.17)
Zea mays var. Sweet corn	a. 19.09 ± 0.52 (25.84)	a.17.08 ± 0.64 (24.35)	a. 13.06 ± 0.24 (21.13)	a. 11.30 ± 0.21 (19.64)	a. 11.30 ± 0.63 (19.64)	a. 8.04 ± 2.05 (24.95)	a. 7.04 ± 0.48 (15.34)	a. 5.78 ± 0.48 (13.81)	a. 4.52 ± 0.41 (12.25)	a. 2.7 ± 0.06 (9.46)
	b. 3.69 ± 1.03 (0.57)	b. 5.52 ± 2.13 (0.74)	b. 4.43 ± 1.19 (0.69)	b. 4.63 ± 1.83 (0.67)	b. 1.69 ± 0.25 (0.23)	b. 2.66 ± 0.45 (0.42)	b. 2.9 ± 0.40 (0.41)	b. 2.5 ± 0.50 (0.39)	b. 1.25 ± 0.19 (0.09)	b. 1.2 ± 0.20 (0.08)

a = Relative lenghth ± SE ( ) = Angular transformation from % to degree.
b = Long arm/short arm ± SE ( ) = Logarithmic Scale (Long arm/Short arm).
(The chromosome numbers have been named from 1-10 in accordance with their length in different varieties).

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Table 2 : K-value of different varieties of *Zea mays* L..

Varieties under consideration	Chromosome 1	Chromosome II	Chromosome III	Chromosome IV	Chromosome V	Chromosome VI	Chromosome VII	Chromosome VIII	Chromosome IX	Chromosome X
Zea mays var. Pop corn, V_1	4.76	24.39	14.70	4.34	11.23	3.44	2.70	10	5	4.34
Zea mays var. KH-9451, V_2	5.88	1.26	2.38	4.16	5.55	3.70	2.32	2.04	7.14	5
Zea mays var. Shanker, V_3	5.26	2.17	1.43	1.69	2.78	2.70	3.44	3.33	5.56	6.66
Zea mays var. K-51, V_4	3.51	2.07	2.22	4.76	2.38	2.77	5.55	5.55	4	24.38
Zea mays var. DMH-849, V_5	2.13	1.64	2.17	7.69	2.86	2.56	3.85	3.70	6.66	6.66
Zea mays ya var. Baby corn, V_6	2.13	1.69	2.17	2.08	2.38	2.77	2.38	4.54	5	6.66
Zea mays var. K-99, V_7	3.57	2.44	1.82	2.17	7.14	8.33	5	5	4	0
Zea mays var. Proagro-4212, V_8	4.54	3.03	5.88	11.11	5.26	2.13	20	4.76	4.99	5
Zea mays var. Super Gold-651, V_9	1.85	3.12	1.41	14.23	5.88	3.12	3.57	2.95	14.29	3.85
Zea mays var. Ganga safed-2, V_{10}	2.70	1.76	4.54	3.85	3.89	1.89	3.23	2.56	5	9.09
Zea mays var. Sweet corn, V_{11}	1.75	1.35	1.45	1.49	4.35	2.38	2.44	2.56	11.12	12.51

Table 3 : Generalised distance and clustering pattern in different varieties of *Zea mays* L..

Sl. No.	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}
1.	$V_{6-727.51}$	$V_{3-24.11}$	$V_{2-24.11}$	$V_{11-228.63}$	$V_{10-31.34}$	$V_{3-30.07}$	$V_{2-85.72}$	$V_{5-306.59}$	$V_{5-31.47}$	$V_{5-31.47}$	$V_{10-67.92}$
2.	$V_{7-743.7}$	$V_{6-43.12}$	$V_{6-30.07}$	$V_{10-257-24}$	$V_{6-37.48}$	$V_{5-37.48}$	$V_{3-106.02}$	$V_{10-367.3}$	$V_{2-177.27}$	$V_{3-41.61}$	$V_{3-66.44}$
3.	$V_{10-784.23}$	$V_{5-43.28}$	$V_{10-41.61}$	$V_{6-335.27}$	$V_{2-43.28}$	$V_{2-43.73}$	$V_{6-108.73}$	$V_{2-392.6}$	$V_{10-243.77}$	$V_{2-47.12}$	$V_{6-87.02}$
4.	$V_{2-788.88}$	$V_{10-47.12}$	$V_{5-72.74}$	$V_{5-338.26}$	$V_{3-72.74}$	$V_{11-87.02}$	$V_{5-139.3}$	$V_{9-395.12}$	$V_{11-254.74}$	$V_{11-67.92}$	$V_{5-98.81}$
5.	$V_{3-798.89}$	$V_{7-85.72}$	$V_{11-86.44}$	$V_{3-376.01}$	$V_{11-98.81}$	$V_{7-108.73}$	$V_{10-236.36}$	$V_{3-395.51}$	$V_{6-260.83}$	$V_{6-171.04}$	$V_{2-100.73}$
6.	$V_{5-811.8}$	$V_{11-100.73}$	$V_{7-106.02}$	$V_{2-425.42}$	$V_{9-121.77}$	$V_{10-171.04}$	$V_{11-267.82}$	$V_{6-424.83}$	$V_{7-304.75}$	$V_{7-236.36}$	$V_{4-228.63}$
7.	$V_{9-900.31}$	$V_{9-117.27}$	$V_{9-313.77}$	$V_{9-596.64}$	$V_{7-139.3}$	$V_{9-260.83}$	$V_{9-304.75}$	V_{7-463}	$V_{3-131.77}$	$V_{9-243.77}$	$V_{9-267.74}$
8.	$V_{11-930.79}$	$V_{9-392.6}$	$V_{4-376.01}$	$V_{8-650.36}$	$V_{8-306.59}$	$V_{4-335.27}$	V_{8-463}	$V_{11-530.61}$	$V_{8-395.12}$	$V_{4-257.24}$	$V_{7-267.82}$
9.	$V_{8-943.59}$	$V_{4-425.4}$	$V_{8-395.57}$	$V_{7-655.52}$	$V_{4-338.26}$	$V_{8-424.83}$	$V_{4-655.52}$	$V_{4-650.36}$	$V_{4-596.64}$	$V_{8-367.3}$	$V_{8-530.61}$
10.	$V_{4-1164.11}$	$V_{1-788.88}$	$V_{1-798.89}$	$V_{1-1164.11}$	$V_{1-811.8}$	$V_{1-727.51}$	$V_{1-743.7}$	$V_{1-943.59}$	$V_{1-900.23}$	$V_{1-784.23}$	$V_{1-930.79}$

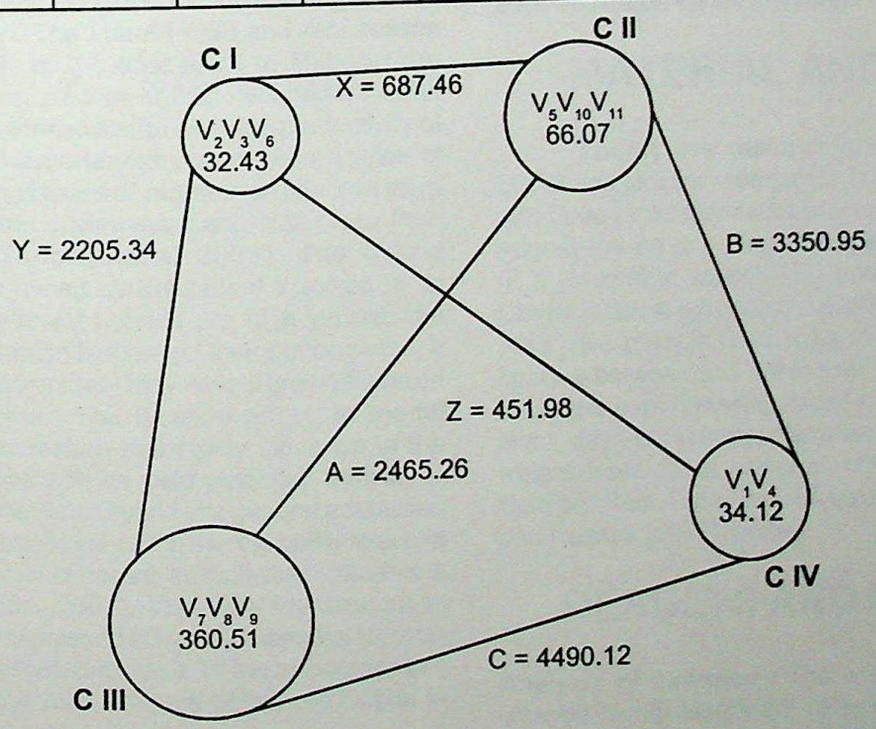

Fig 1 : Generalised distance between and within constellations on the basis of D^2 - value among eleven varieties of *Zea mays* L.

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(The chromosome numbers have been named from 1-10 in accordance with their length in different varieties)

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Singh *et. al* (2006), Kumar (2008) and Rani and Kumar (2010). The D^2 statistical finding could be exploited in hybridization programme as they are expected toe produce good heterotic effect for grain yield.

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Stability Analysis for Pollen Production and Yield Components in Restorer Lines of Sunflower (*Helianthus annuus* L.)

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Twenty five restorer lines were evaluated for their stability analysis for pollen production and yield components in six environments. The analysis of variance for stability revealed that the mean sun of squares due to environment linear was highly significant indicating that a major part of variation could be attributed to linear regression. The significant variance due to pooled deviation for characters days to 50% flowering, plant height, pollen production, days to maturity, seed filling per cent, hull content per cent and seed yield per plant indicated that differences in stability for these traits were due to high linear (b) and non-linear (S^2d) parameter. The studies on estimates of stability parameter revealed that the restorer line NDR-2, NDR-7-IB, J/6 for pollen production, harvest index, test weight, low hull content , oil content per cent, NDR-273 for seed filling dwarf plant height, head diameter and oil content, LR-06-3, LR-6-12 and LTR-08-7 for early maturity, seed yield and head diameter, NDLR-06 for seed filling and oil content and DMLT-1Y for seed yield and harvest index were observed with average stability and are expected to be better in all environments.

Keywords : Stability analysis, Regression coefficient, Sunflower, G x E interaction.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important oil seed crop in the world. The relative thermo and photo-insensitivity of sunflower allows its cultivation throughout the year in different season. The *kharif* (40%) and *rabi* season (52%) account for 92% of the total area. In Maharashtra sunflower is cultivated on an area of 2.50 lakh hectares with production of 1.54 lakh tones with an average productivity of 628 Kg/ha. *Kharif* corp experiences one or more cycles of moisture stress. In contrast to *kharif*, *rabi* season provides more favorable and predictable environment and suffer less from pest and diseases (Anonymous, 2009). The hybrid performance was determined by the parent involved in its synthesis. Though sunflower hybrids are high yielder, the influence of G x E interaction is more on their performance, a specific difference in environment may have a great effects on some genotype the other. The location effect, seasonal fluctuation and their interaction has highly influence in the performance of genotype with its yield potential. Stability is essential in breeding varieties or hybrids for general adaptation particularly for crop like sunflower which has the ability to adjust and grow successfully in different agroclimatic situations (Damodaran and Hegde, 2007). The diversity present in parental lines would get expressed in hybrids. Attention towards high yield potential and other attributes in 'R' line as not received adequate attention in the past. Profuse pollen production in male lines in important requirement for both seed production and commercial crop production. Evolution of sunflower restorer line with stable pollen production in different growing season will be highly useful to seed production agencies to get consistent yield. Thus twenty five sunflower restorer lines were evaluated for six environments with above aim.

MATERIAL AND METHODS

Twenty five restorer lines including 2 checks *viz.* LSF-8 and Morden was grown in a randomized block design with three replication in six environment *viz.* July sowing (E_1), August sowing (E_2), September sowing (E_3), October sowing (E_4), November sowing (E_5) and December sowing (E_6) at Oilseeds Research Station, Latur during 2009-10. The restorer lines were sown in three rows of 3 m length with 60 x 30 cm spacing between and within row. Data were recorded on days to 50 per cent flowering, plant height, pollen production per plant, days to maturity, diameter of head, harvest index, test weight, seed filling per cent, hull content, oil content, seed yield per plant. Data were analyzed for stability as per Eberhart and Russell (1966) model.

RESULTS AND DISCUSSION

Analysis of variance : The analysis of variance (Table-1) showed highly significant differences due to genotype and

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environments indicating presence of variation among genotype as well as among environments. The significance of G x E (linear) for component days to 50% flowering, plant height, days to maturity, head diameter, harvest index, 100 seed weight, seed filling (%) and oil content (%) indicates that the variation in the performance of genotype is due to the regression of genotype on environments and hence the performance is predictable in nature.

Phenotypic stability : The phenotypic stability was assessed by considering the mean performance of the genotype over environments, the linear regression of genotypes over environment index (bi) and the diviation from this regression (S^2di). The stable genotype is that one which have high mean, regression coefficient (bi) equal to unity and deviation from regression (S^2di) as small as possible or approaching zero. Twenty five restorer lines including 2 checks *viz.* LSF-8 and Morden were tested with three replication over six environment *viz.* July sowing (E_1), August sowing (E_2), September sowing (E_3), October sowing (E_4), November sowing (E_5) and December sowing (E_6) at Oilseeds Research Station, Latur. Effect of sowing time on yield and other traits presented in Table 3 revealed that significant differences noticed for all the seasons as well as restorers. Among the six environments, the October sowing was found superior for most of the yield attributes.

The regression analysis (Table 2) showed that the mean sum of squares due to environment (linear) indicating a major part of variation could be attributed to linear regression. The significance of G x E interactions (linear) for days to 50% flowering, plant height, head diameter, days to maturity, seed filling %, and oil content indicates that the variation in the performance of genotype is due to the regression of genotype on environments and hence the performance in predictable in nature. Mean squares due to pooled deviation were found significant for all the characters except head diameter, harvest index 100 seed weight and oil content indicate the greater role of unpredictable components in genotype x environment interaction. These findings are in agreement to those of Due and Yadava (1981), Singh and Yadava (1982), Choudhary and Anand (1988), Xanthopoulos (1994). The non-linear component of G x E interaction was found significant in restorer line such as P-35R, LOC-6443, R-274, LTR-08-06, SH-41-1 and MR-234 for days to 50% flowering suggestion that restorer lines differ considerably with respect to stability of flowering. The restorer line NDLR-06, IBNT-05, 83R6 NDR-2 and J-6 were of average stability for days to 50% flowering. As far as days to maturity are concerned the restorer lines *viz.*, LTR-08-07, NDR-83R6, LR-06-12, NDR-2 and LR-06-03 could fulfilled the requirements of average stability for days to maturity.

In respect of pollen productions the restorer lines SH-41-1 and 6D-IR recorded above average stability while NDR-7-1B, J/6, 83R6, NDR-83R6 and check Morden recorded greater stability because it possess high mean pollen production, regression coefficient towards unity and non-significant least S^2di value. NDR-2, LR-06-03, MR-234 recorded below average stability while above average stability were noticed by R-298 and DMLT-1Y for filled seed per cent. The

Table 1 : Analysis of variances for G x E interaction.

Source of variation	d.f.	Days to 50% Flowering	Plant Height (cm)	Pollen production /plant (gm)	Days to maturity	Head diameter (cm)	Harvest index (%)	100 Seed weight (gm)	Seed filling (%)	Hull content (%)	Oil content (%)	Seed yield/plant (gm)
		Mean sum of Squares										
Genotype	24	64.32**	597.78**	0.0344**	42.99**	14.85**	31.44**	2.44**	29.84**	94.84**	29.33**	164.22**
Environment	5	107.80**	1805.91	0.265**	86.98**	34.33**	6.77**	0.23**	122.64**	92.98**	114.93**	55.68**
G X E	120	4.42**	127.68**	0.006	5.60**	2.02**	1.91	0.10	3.49**	3.16**	2.91	3.47**
E + (G X E)	125	8.55	194.81	0.007	8.86	3.31	2.10	0.11	8.26	3.40	7.40	5.56
E (Linear)	1	538.60	9029.99**	0.13**	434.55**	171.67**	33.92**	1.18**	613**	46.46**	574.58**	278.48**
G X E (Linear)	24	5.44**	186.06*	0.002	9.18**	4.25**	2.86**	0.103*	6.94**	2.91	6.61**	2.89
Pooled deviation	100	4.00**	108.56**	0.007**	4.53**	1.40*	1.60	0.103**	2.53**	3.09**	1.91*	3.47**
Pooled error	288	1.61	6.00	0.007	1.68	2.11	3.16	0.129	2.08	1.61	3.007	3.16

*,** Significant at 5% and 1% level with tested against pooled deviation respectively.

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Table 2 : Estimates of stability parameters in restorer lines under six environment.

S. No.	Restorer	Days to 50 % flowering			Plant height (cm)			Pollen production/plant (g)			Days to maturity		
		Mean	bi	S^2di	Mean	bi	S^2di	Mean	bi	S^2di	Mean	bi	S^2di
1.	NDR-7-1B	57.94	1.55	3.52**	115.59	0.62	61.55**	0.65	1.07	0.00	90.33	1.73	15.02**
2.	NDR-273	60.67	1.37	4.00**	122.05	1.16	3.73**	0.60	1.52	0.03**	91.17	1.51	0.94**
3.	NDR-7-90	57.28	1.45	2.66**	116.68	0.79	81.66**	0.56	1.34	0.01**	90.00	0.35	2.19**
4.	LTR-08-07	64.39	0.10	0.43	147.43	1.59	218.75**	0.58	1.53	0.01**	90.00	0.35	2.19**
5.	R-298	61.06	1.70	0.24	107.52	0.24	182.18**	0.36	0.30	0.02**	92.89	0.66	4.71**
6..	6D-1R	66.22	0.40	1.63**	114.89	0.58	19.38**	0.32	-0.23	0.00	95.50	1.68	4.04**
7.	J/6	67.22	0.81	-0.30	115.96	0.26	54.26**	0.53	1.08	0.00	94.44	-0.05	4.63**
8..	83R6	60.28	1.52	0.01	117.89	1.34	290.19**	0.50	0.79	0.00	90.33	1.51	3.45**
9.	RHA-1-1	65.61	0.65	1.82**	118.66	1.64	46.24**	0.44	0.67	0.02**	95.83	1.74	1.60**
10.	AK-345	62.83	0.41	2.83**	147.42	1.47	158.84**	0.49	0.53	0.01**	91.67	-0.20	3.48**
11.	P-35R	59.17	0.78	16.98**	115.46	0.79	51.60**	0.43	0.44	0.01**	90.56	0.03	16.08**
12.	NDLR-06	58.89	0.85	-0.06	113.48	0.40	124.01**	0.45	1.77	0.00	88.06	-0.33	4.26*8
13.	LOC-6443	59.39	1.21	12.08**	126.35	1.41	443.72**	0.47	0.86	0.00	90.44	0.98	7.43**
14.	IBNT-05	58.33	0.61	-0.31	129.09**	1.82	121.09**	0.47	1.49	0.00	89.83	2.40**	0.41
15.	NDR-83R6	58.28	1.08	1.04**	118.37	0.01	74.82**	0.52	0.99	0.00	90.89	1.93	0.75
16.	LTR-08-06	62.11	0.96	5.49**	122.65	1.54	28.73**	0.46	0.75	0.00	92.67	1.62	7.08**
17.	R-274	63.72	1.00	9.44**	124.37	1.09	246.56**	0.42	1.25	0.01**	93.78	1.04	2.97**
18.	LR-06-12	65.89	-0.02	0.77	135.59	2.10*	121.56**	0.52	0.04	0.01**	92.50	0.80	0.57
19.	SH-41-1	56.17	1.50	5.17**	121.48	1.40	102.38**	0.40	0.33	0.00	87.67	0.62	1.71**
20.	NDR-2	59.56	1.11	0.23	111.70	2.25*	39.76**	0.47	1.34	0.00	89.67	0.80	-0.25
21.	LR-06-03	63.44	1.65	4.61**	121.70	1.39	141.81**	0.45	0.65	0.00	91.44	1.34	0.87
22.	MR-234	59.94	0.87	5.68**	111.55	1.25	9.87**	0.48	1.83	0.00	88.72	0.61	9.96**
23.	DMLT-1Y	56.28	0.39	1.90**	112.28	0.11	2.28	0.61	2.85	0.02**	83.83	0.36	1.15**
24.	LSF-8 (c)	62.11	1.50	3.70**	124.02	0.46	16.54**	0.49	1.01	0.00	92.94	0.98	4.78**
25.	MORDEN (c)	56.78	1.57	3.15**	121.48	-0.73	19.49**	0.50	0.79	0.00	89.11	1.78	1.17**
S.E.			0.89			4.65				0.039		0.95	
S.E. (bi)			0.43			0.54				1.20		0.51	

average stability for seed filling over all environments was found in NDR-273, AK-345 and NPLR-06. The restorer lines NDR-7-1B, NDR-7-90, J/6, LR-06-3, MR-234 and DMLT-1Y showed average stability for overall environment for harvet index.

The restorer line J/6, NDR-273, NDR-7-1B, 6D-1R, NDLR-06, IBNT-05 and LSF-8 had regression coefficient towards unity and non-significant S^2di associated with high oil content over grand mean could be considered as stable restorer line and widely adoptable where as SH-41-1, NDR-2, MR-234, DMLT-1Y and RHA-1-1, AK-345 exhibited above average and below average stability respectively. Seetaram *et al.*, (1980) also reported that sunflower hybrids in general had greater specificity of adaptability to favorable environmental conditions. The lines LTR-08-6, LR-06-12, LR-06-3, DMLT-1Y and LTR-08-7 were found to be ideal for growing over a wide range of environments for seed yield per plant.

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S. No.	Restorer	Head diameter (cm)			Harvest index (%)			100 Seed weight (g)			Seed filling (%)		
		Mean	bi	S²di	Mean	bi	S²di	Mean	bi	S²di	Mean	bi	S²di
1.	NDR-7-1B	14.12	0.91	2.87**	31.91	1.70	-0.52	4.51	0.99	-0.03	78.19	1.36	-0.62
2.	NDR-273	14.51	1.33	-0.40	31.61	0.51	3.30*	3.86	2.84	0.06	80.83	1.06	0.56
3.	NDR-7-90	14.64	1.30	0.65	31.15	2.88	0.30	4.63	-0.58	0.06	80.32	1.59	4.31**
4.	LTR-08-07	15.27	1.74	-0.40	30.31	1.48	0.65	5.32	-2.10	0.19**	81.09	-0.05	6.05**
5.	R-298	11.40	0.40	4.04**	28.50	1.10	-0.18	3.34	1.45	-0.03	77.77	0.36	0.83
6.	6D-1R	11.38	1.64	3.05**	27.15	0.80	-0.04	4.37	-1.11	0.13**	80.28	0.02	1.64**
7.	J/6	15.94	0.74	0.15	32.78	1.24	-0.71	4.77	2.26	-0.03	80.74	1.50	1.54**
8.	83R6	13.25	0.06	0.10	25.47	0.92	-0.57	4.10	0.32	0.03	76.11	1.17	1.09*
9.	RHA-1-1	9.07	-0.03	-0.57	25.26	-0.45	1.90*	3.33	0.20	0.01	74.88	1.55	0.94
10.	AK-345	13.96	1.96*	1.04	26.86	1.15	-0.03	3.84	2.65	0.02	81.48	1.32	0.49
11.	P-35R	13.77	1.97*	0.54	26.09	-2.77	0.63	4.04	0.55	0.13**	75.78	1.02	0.18
12.	NDLR-06	14.42	1.56	1.86**	27.43	-1.00	-0.12	4.41	1.22	0.16**	79.87	0.99	-0.05
13.	LOC-6443	13.60	0.53	0.47	26.85	1.07	0.05	4.84	0.49	0.06	78.31	0.78	3.85**
14.	IBNT-05	15.11	0.10	-0.14	26.72	-2.09	1.98**	4.21	2.56	0.09**	82.26	0.86	6.79**
15.	NDR-83R6	14.62	2.24**	1.19*	28.00	0.24	2.60**	5.02	-0.43	-0.02	77.59	0.71	2.63**
16.	LTR-08-06	14.59	1.60	0.59	30.10	0.42	3.08**	4.07	2.19	-0.01	77.84	0.78	2.15**
17.	R-274	10.99	0.14	-0.45	26.16	2.07	5.02**	4.11	-2.30	0.25**	74.36	1.00	0.35
18.	LR-06-12	14.90	0.51	0.07	27.01	2.46	-0.88	5.66	2.50	0.00	81.06	0.40	3.28**
19.	SH-41-1	11.73	1.90*	-0.22	27.13	1.47	-0.72	5.30	2.07	0.12**	78.24	1.19	-0.27
20.	NDR-2	12.99	2.00*	0.01	28.81	0.48	-0.48	3.94	1.35	-0.03	78.86	1.76*	1.49**
21.	LR-06-03	13.92	1.60	0.87	32.11	1.50	-0.96	4.48	2.78	-0.04	77.52	1.90**	1.04
22.	MR-234	13.22	0.75	0.27	31.60	1.39	-0.41	4.66	0.47	0.09**	80.26	1.77*	1.98**
23.	DMLT-1Y	13.06	-0.14	-0.22	30.00	1.89	0.24	5.89	0.82	0.30**	78.86	0.83	3.89**
24.	LSF-8 (c)	13.57	0.19	1.75*	29.20	3.35*	0.28 4.53	2.53	2.48	0.02	79.86	0.83	3.89**
25.	MORDEN (c)	12.60	0.00	0.41	30.00	3.19*	-0.58	4.61	1.34	-0.04	82.89	0.86	2.22**
S.E.			0.53			0.56			0.14				0.71
S.E. (bi)			0.45			1.08			1.47				0.32

S.No.	Restorer	Hull content (%)			Oil content (%)			Seed yield per plant (g)		
		Mean	bi	S²di	Mean	bi	S²di	Mean	bi	S²di
1.	NDR-7-1B	25.29	1.66	0.22	34.95	0.77	0.16	21.81	1.20	1.75
2.	NDR-273	21.35	0.14	0.37	35.86	1.31	0.62	27.57	0.07	0.43
3.	NDR-7-90	30.64	1.16	0.86	32.38	0.79	1.78	23.09	1.43	3.86
4.	LTR-08-07	31.52	3.07	0.17	34.35	0.28	10.16	27.90	0.68	-0.46
5.	R-298	29.61	0.18	0.62	31.05	0.95	0.27	17.27	0.22	0.05
6.	6D-1R	30.07	1.89	0.07	33.87	1.13	0.71	18.40	1.58	7.51
7.	J/6	30.15	1.14	6.27	35.66	0.86	-0.32	22.39	0.31	0.21
8.	83R6	34.05	2.99	4.48	31.27	1.44	0.96	20.88	0.89	3.89
9.	RHA-1-1	21.89	-0.26	1.48	32.79	1.85	0.63	13.52	0.67	-0.42
10.	AK-345	24.34	0.90	0.89	35.87	2.26	0.58	24.43	0.48	1.59
11.	P-35R	29.62	0.06	4.07	31.04	1.45	1.62	25.29	1.52	6.96
12.	NDLR-06	29.91	1.66	1.74	34.43	1.16	0.74	28.80	0.71	5.30
13.	LOC-6443	34.73	1.15	1.91	33.57	1.37	-0.65	26.43	1.56	8.65

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14.	IBNT-05	31.97	0.10	3.03	34.07	1.39	-0.60	27.25	0.45	5.12
15.	NDR-83R6	34.37	0.02	2.89	31.26	1.42	-0.44	24.94	1.46	2.72
16.	LTR-08-06	34.84	1.12	3.23	31.90	1.19	-0.11	32.26	1.06	-0.18
17.	R-274	26.74	3.06	9.64	33.58	1.00	2.47	15.16	1.42	1.52
18.	LR-06-12	29.38	1.79	1.16	33.32	1.28	-0.94	31.18	0.72	1.24
19.	SH-41-1	36.81	0.48	1.04	30.06	0.51	0.14	23.39	1.83	2.67
20.	NDR-2	34.65	-0.23	2.74	30.83	0.30	-0.18	29.65	1.40	1.85
21.	LR-06-03	28.74	0.67	3.36	34.88	0.56	2.85	29.42	1.01	0.41
22.	MR-234	32.47	0.50	3.22	34.05	0.52	-0.84	24.87	1.63	4.29
23.	DMLT-1Y	31.92	2.33	7.16	39.8	0.18	-0.05	31.60	0.33	0.30
24.	LSF-8 (c)	29.86	1.94	2.69	34.57	1.04	0.85	18.91	1.06	1.05
25.	MORDEN (c)	32.50	-2.51	0.64	30.67	0.00	2.45	17.75	1.31	0.13

Table 3 : Grand Mean performance of restorer lines for yield attributes in six environments.

S.No.	Characters	Environments						Pooled mean
		E-1	E-2	E-3	E-4	E-5	E-6	
1.	Pollen production/plant (g)	0.46	0.50	0.46	**0.54**	0.46	0.50	0.50
2.	Head diameter (cm)	13.56	14.64	15.08	**12.70**	12.29	12.52	13.47
3.	100 seed weight (g)	4.31	4.59	4.49	**4.53**	4.41	4.51	4.47
4.	Seed filling (%)	76.61	78.30	80.56	**81.56**	80.61	76.40	79.01
5.	Hull content (%)	30.46	30.75	30.24	**29.15**	30.34	30.84	30.30
6.	Oil content (%)	33.86	34.26	34.24	**34.78**	34.41	29.11	33.44
7.	Harvest Index (%)	27.98	28.46	29.26	**29.38**	28.59	28.71	28.73
8.	Seed yield per plant (g)	22.03	23.87	24.22	**26.68**	24.13	23.84	24.17

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Genetic Variability Studies in Cassava (*Manihot esculenta* Crantz)

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A field evaluation of 361 cassava germplasm accessions was carried out from 2006-2009 at Tapioca and Castor Research Station, Yethapur in Tamil Nadu. The characters *viz*, plant height, stem girth, number of tubers per plant, tuber girth, yield per plant and starch content showed significant difference among the varieties. The phenotypic and genotypic variance were highest for plant height and tuber length. The heritability estimates ranged from 2.80 to 41.38 per cent. The correlation studies revealed that the component characters had a positive and significant association with root yield.

Keywords : Cassava, Variability, Heredity and Correlation co-efficient.

INTRODUCTION

In cassava (*Manihot esculenta* Crantz), a large number of cultivars are in existence in the major cassava growing countries of the world. These cultivars vary widely in the growth, yield and quality character. For basic breeding programme, the study of genetic variability of the population and the pattern of the correlation among the quantitative trait is more important. The present study was made to collect the information available on the subject particularly in regard to heritability and correlation of yield and tuber characters in cassava.

MATERIAL AND METHODS

The study was carried out in the germplasm collection comprising of 361 germplasm accessions. The data in respect of seven character were recorded on individual plant basis from a population of ten plants in each cultivar. Phenotypic and genotypic variances were calculated following Lush (1949). The co-efficient of variability were computed after Burton (1952). Heritability in the broad sense were calculated based on the methods of Johnson *et al.*, (1955). The simple correlation co-efficients between tuber yield and the other component characters were worked out following Panse and Sukhatme (1957).

RESULTS AND DISCUSSION

Variability and Heritability : The range, mean, the phenotyic and genotypic variances, the estimates of phenotypic and genotypic co-efficient of variability, heritability are presented in Table 1. The results showed that there was a highly significant difference among the varieties in respect of plant height, stem girth, number of tubers per plant, girth of tuber, yield per plant and starch content. The variability was higher in respect of yield as observed from co-efficient of variability followed by number of tubers per plant.

The other characters showed lesser magnitude of variability, with the co-efficient of variability ranging from 6.61 to 45.87. The starch content showed the least co-efficient of variability followed by tuber girth. Balashanmugham *et al.*, (1980) also observed least co-efficient of variability for tuber grith. Manugu *et al.*, (1984) observed low co-efficient of variability for stem girth.

The phenotypic variance as well as the genotypic variance was highest for plant height followed by tuber length. These parameters were low for tuber yield per plant. The highest phenotypic co-efficient of variation was seen in respect of tuber yield per plant followed by number of tubers. Vast phenotypic variability was reported in the CIAT germplasm (Kawano kazno, 1975).

The genotypic co-efficient of variation also presented the same trend as the phenotypic co-efficient of variation with tuber yield and number of tubers per plant exhibiting higher magnitudes of variation than the other characters. Similarly, relative large phenotypic and genotypic co-efficient of variation were observed for storage root yield per plant of 36 germplasm accessions of cassava by Amsalu Nebiyu (2006).

The heritability estimates ranged from 2.80 to 41.38 per cent thus revealing high heritability for the characters studies. The heritability was highest for starch content (41.38) followed by number of tubers per plant (30.9). The heritability for yield was low whereas Barry Nestel and James Cock (1976) in his genetic studies observed high heritability for root size and shape and low heritability for yield.

The present study showed high heritability for starch content, number of tubers per plant and plant height whereas Mahungu *et al.*, (1984) observed moderately high heritability

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Table 1 : Estimates of genetic variability in Cassava (*Manihot esculenta* Crantz).

Characters	Mean	Range	CV	Variance		PCV	GCV	h^2 (per cent)
				Phenotypic	Genotypic			
Plant height (cm)	196.4	106.3 - 314.8	20.33	2096.7	506.48	23.35	11.48	24.0
Stem girth (cm)	6.8	3.8 - 9.8	20.14	2.23	0.33	21.83	8.40	15.0
Number of tubers per plant	5.3	2.3 - 8.4	36.04	3.88	0.19	36.96	8.39	30.9
Mean Tuber length (cm)	24.6	13.8 - 38.8	23.50	34.28	0.96	23.83	3.99	2.80
Mean Tuber girth (cm)	17.04	9.07 - 23.93	17.67	10.73	1.75	19.30	7.80	16.3
Tuber yield per plant (Kg)	1.58	0.72 - 3. 79	45.87	0.56	0.04	47.49	12.34	6.75
Starch content (%)	15.8	11.3 - 25.2	6.61	4.06	1.68	8.63	5.55	41.38

Table 2 : Single correlation co-efficients among seven characters.

Sl.	Characters	X1	X2	X3	X4	X5	X6	X7
X1	Tuber yield per plant (Kg) (X1)	-	0.139	0.247	0.475	0.212	0.229	0.085
X2	Plant height (cm) (X2)		-	0.523	0.122	0.028	0.237	0.024
X3	Stem girth (cm) (X3)			-	0.267	0.054	0.227	0.032
X4	Number of the tubers per plant (X4)				-	0.012	0.133	0.045
X5	Mean tuber length (cm) (X5)					-	0.009	0.014
X6	Mean tuber girth (cm) (X6)						-	0.054
X7	Starch content (%) (X7)							-

** Significant at 1 per cent level

(50%) for root yield and number of roots. The stem girth, tuber girth, tuber length, tuber yield recorded moderate to low heritability from 24 to 6.75 per cent. This finding coincides with the findings of Mahungu *et al.,* (1984). Number of tubers per plant and plant height may be considered while selection programme because of high heritability.

Correlation among characters : The results are tabulated in Table 2. It was found that the component characters had a positive and significant association with root yield. Tuber number had positive association with yield. Similarly Magoon *et al.,* (1972) and Shanmugham *et al.,* (1974) also found positive association between tuber number and yield. Asante and Dixon (2002) observe highly significant positive correlation ranging from 0.73 to 1.00 for root number, root weight and fresh yield.

Therefore emphasis should be given on tuber number, stem girth, tuber girth, length and plant height while selecting a good genotype for enhancing cassava yield. In this study, the tuber yield was positively and significantly correlated with plant height and tuber girth. This finding coincides with the findings of Padma *et al.,* (2009).

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Triple Test-cross Analysis for Seed Yield and its Component Characters in Yellow *sarson* (*Brassica campestris* var. Yellow *sarson*)

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The modified triple test-cross analysis was applied to study additive, dominance and epistatic components of genetic variance for 11 quantitative traits in yellow *sarson* during *rabi* season, 2006-07. A triple test-cross set of 60 crosses was derived by crossing 20 lines with three testers, viz., NDYS-2, YST-151 and their hybrid, NDYS-2 x YST-151. The 83 genotypes comprising 40 single crosses, 20 three-way crosses and 23 parents were evaluated in randomized complete block design with three replications. The total epistasis was evident for all the eleven characters under study. The fixable additive x additive (i) type epistasis was significant only for days to maturity and 1000-seed weight, whereas 'j+l' type epistatic component played important role in the expression of all the eleven characters. The additive (D) as well as dominance (H) components of genetic variance were highly significant for all the characters under study, except non-significant dominance variance observed for days to 50% flowering. All traits showed partial dominance except over dominance recorded for 1000-seed weight, harvest-index and oil content. The non-significant values of directional element of dominance (F) for all the traits, except 1000-seed weight and oil content, indicated ambidirectional nature of dominane and alleles with the increasing and decreasing effects appear to be dominant and recessive to the same extent. The negative and significant value of 'F' for 1000-seed weight indicated that the increasing type of genes for this character were dominant in nature, while positive and significant value of this parameter for oil content suggested that contribution towards dominance variance was influenced by higher proportion of decreasing alleles.

Keywords : Additive, Dominance, Epistatic variation, Yellow *sarson.*

INTRODUCTION

The precise knowledge of additive, dominance and epistatic components of genetic variance involved in the expression of important quantitative characters is essential for planning and execution of an effective breeding programme in any crop. However, majority of available reports on analysis of gentic variation in yellow *sarson* (*Brassica campestis* var. yellow *sarson*) are based on the methods of second degree statistics that either assume absence of epistasis or provide narrow cross-wise estimates of epistasis. The modified triple test-cross analysis of Ketata *et al.* (1976) provides efficient and unbiased estimates of additive, dominance and epistatic components of genetic variance using larger sample of crosses. Therefore, an attempt was made to assess the role of various components of genetic variance in the inheritance of eleven quantitative traits in yellow *sarson* using modified triple test-cross analysis (TTC) analysis.

MATERIAL AND METHODS

Two varieties of yellow *sarson*, NDYS-2 and YST-151 and their hybrid (NDYS-2 x YST-151) were crossed as testers with 20 diverse lines, namely, NDYS-128, NDYS-141, NDYS-116-1, NDYS-9503, NDYS-9506, NDYS-9508, RYSK-05-1, BINOY, MYSL-2003-2, NDYS-7, NDYS-17, NDYS-51, MYSL-2005, NDYS-921, NRCY-5, NRCY-7, SSK-92-13, MYSL-2004, RAUDYS-81-9 and BIO-YS-1. The experimental material consisting 3 testers, 20 lines, 40 single crosses and 20 three-way crosses was evaluated in randomized complete block design with three replications during *rabi* season of 2006-07 at Pilikothi Farm of T.D.P.G. College, Jaunpur. The rows of 3m length were used for providing single row plots for parents and crosses and double row plots for three-way crosses following between rows and within rows spacings of 30 cm and 10 cm, respectively. Observations were recorded on five plants each of parents and single crosses and fifteen plants of three-way crosses by tagging randomly selected competitive plants in each plot. The characters means for 11 quantitative traits, *viz.*, days to 50% flowering, days to maturity, plant height, primary branches per plant, siliquae per plant, seeds per

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siliqua, 1000-seed weight, biological yield per plant, seed yield per plant, harvest-index and oil content were subjected to modified triple test-cross analysis of Ketata *et al.* (1976).

RESULTS AND DISCUSSION

The analysis of variance of triple test-cross for test of epistasis revealed significant role of over all epistasis in expression of all the eleven characters under study (Table 1). The partitioning of total epistasis into 'i' and 'j+l' type components showed that additive x additive (i) type epistasis was significant only for days to maturity and 1000-seed weight. The 'j' (additive x dominance) and 'l' (dominance x dominance) type epistasis was significant for all the eleven characters. However, the relative contribution of 'j+l' type epistasis was greater than that of 'i' type for all the traits under study. Therefore, 'j+l' type epistatic component seems to be of greater significance for these traits. The existence of significant epistasis in the inheritance of seed yield and its components in yellow *sarson* was also reported by Tiwari (2007) who observed importance of additive x additive (i) epistasis for plant height, primary branches per plant, length of main raceme and seeds per siliqua and presene of significant dominance x dominance (l) epistasis for primary branches per plant, siliquae per plant, length of main raceme, seeds per siliqua and seed yield per plant.

The additive (D) as well as dominane (H) components of genetic variance were significant for all the characters except non-significant estimate of dominance variance recorded for days to 50% flowering (Table 2). The important role of additive as well as dominance gene effects for seed yield and some other compoent traits in yellow *sarson* was also observed by Labana *et al.* (1978), Verma *et al.* (1989), Singh and Mishra (2001), Tiwari (2007) and Gautam (2008). The higher estimates of H than D for 1000-seed weight, harvest-index and oil content resulted into greater than one value of average degree of dominance indicating over dominance. The lesser than one value of average degree of dominance resulting due to higher estimates of D than H, suggested existence of partial

Table 1 : Analysis of variance for the test of epistasis in tripletest-cross for eleven characters in yellow *sarson*.

Source	d.f.	Days of 50% flowering	Days of maturity	Plant height (cm)	Primary branches per plant	Siliquae per plant	Seeds per siliqua	1000-seed weight (g)	Biological yield per plant (g)	Seed yield per plant (g)	Harvest index	Oil content (%)
i type epistasis	1	340.82	86.40*	1865.95	14.60	45.24	4.87	10.75**	721.76	6.27	88.57	1.60
j + l type epistasis	19	14.06*	23.84*	674.84**	12.54**	567.40**	58.94**	3.02**	272.24**	10.28**	61.78**	19.27**
Total epistasis	20	27.55**	26.97*	734.40**	12.64**	541.29**	56.24**	3.41**	294.72**	10.08**	63.12**	18.39**
i type epistasis x blocks	2	85.20**	21.60	466.49**	3.65**	11.31	1.22	2.69	180.44**	1.57	22.14**	0.40
j+l type epistasis x blocks	38	6.12	12.09	11.92	0.30	17.51	4.40	6.10	5.69	1.42	4.79	0.52
Total epistasis x blocks	40	10.07	12.57	34.65	0.47	17.20	4.24	0.19	14.43	1.42	5.65	0.52

* Significant at 5% probability level,
** Significant at 1% probability level.

Table 2 : Estimates of additive (D) and dominance (H) variance components, average degree of dominance $(H/D)^{0.05}$ and direction of dominance (F) for eleven characters in yellow *sarson*.

Characters	Genetic parameters			
	D	H	$(H/D)^{0.5}$	F
Days to 50% flowering	133.47**	1.54	0.107	-0.308
Days to maturity	131.16**	25.86**	0.444	0.335
Plant height (cm)	2409.45**	817.87**	0.583	1.361
Primary branches per palnt	12.80**	11.09**	0.931	0.189
Siliquae per plant	3203.94**	340.37**	0.326	0.204
Seeds per siliqua	346.23**	28.93**	0.289	0.029
1000-seed weight (g)	1.77**	2.25**	1.127	-3.115*
Biological yield per plant (g)	730.03**	443.07**	0.779	0.143
Seed yield per plant (g)	29.23**	6.97**	0.488	0.317
Harvest index	44.84**	59.06**	1.148	-0.191
Oil content (%)	18.33**	19.83**	1.040	8.249**

* Significant at 5% probability level,
** Significant at 1% probability level.

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dominance for remaining eight characters. The non-significant values of directional element of dominance (F) for all the characters under study, except 1000-seed weight and oil content, revealed presence of ambidirectional dominance and alleles with increasing and decreasing effects appear to be dominant and recessive to the same extent. The negative and significant value of 'F' for 1000-seed weight indicated that the increasing type of genes for this character were dominant in nature, while positive and significant value of this parameter for oil content suggested that contribution towards dominance variane was influenced by higher proportion of decreasing alleles.

The existence of highly significant additive genetic variance of fixable nature for seed yield per plant as well as remaining ten component traits indicated that progress through selection could be expected in these traits even in early segregating generations. Thus, substantial improvement in seed yield and its components can be achieved in yellow *sarson* by employing the conventional breeding procedures aimed at development of high yielding pureline varieties. Further improvement through selection seems possible in case of days to maturity and 1000-seed weight, if selection is delayed to later generations to capitalize upon the fixable genetic variability resulting from significant additive x additive (i) epistatic component. Similar conclusion was also drawn by Ram Bhajan *et al.* (1994) in Indian mustard. The results also revealed the significance of dominance variance (H) and additive x dominance (j) and dominance x dominance (l) type epistasis for all the characters except non-significant 'H' for days to 50% flowering. This suggested predominant role of non-fixable and non-additive gene effects represented by significant 'H' and 'j+l' components which can be exploited through heterosis breeding. Being an autogamous crop, where commercial exploitation of hybrids has not yet started, the non-fixable components of genetic variation have limited use in yellow *sarson*. However, the non-additive gene effects may be exploited to some extent for improving the characters by resorting to breeding methods such as biparental mating followed by recurrent selection and population improvement methods as suggested by Jensen (1970) and Redden and Jensen (1974).

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Genetic Variability, Heritability and Genetic Advance for Yield and its Contributing Traits in Cabbage (*Brassica oleracea* var. *capitata* L.)

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The studies on genetic variability, heritability, genetic advance were carried out in 30 diverse genotypes of cabbage (*Brassica oleracea* var. *capitata* L.) for 11 quantitative characters. The study showed high range for yield (11.10 – 56.60), days to maturity (71.28 – 110.53) and leaf length (15.95 – 46.02). The highest estimates of PCV and GCV were for stalk length (PCV = 40.42, GCV = 39.21) followed by core length (PCV = 37.43, GCV = 36.02) and yield (PCV = 37.07, GCV = 36.87) and low for leaf width (PCV = 12.51, GCV = 21.12) followed by polar length (PCV = 16.85, GCV = 16.47) and days to maturity (PCV = 12.91 , GCV = 11.50). Narrow differences between PCV and GCV gave evidence to the lines that the variability exiting in them was mainly due to their genetic make up. High estimates of heritability was noticed for yield (98.91%) followed by leaf length (97.30%) and equatorial length (97.10%). Number of non-wrapper leaves, head weight, core length and stalk length showed moderate heritability estimates. Days to maturity (79.40%) and gross weight (78.60%) showed low heritability estimates. High heritability in broad sense indicated that large proportion of phenotypic variance was attributable to the genotypic variance and were less influenced by environment. High % of genetic gain was observed for yield (75. 54%) whereas it was low for days to maturity (21.10%) and polar length (33.16%). Hence, selection can bring worth while improvement in these traits.

Keywords : Genetic Variability, Heritability, Genetic Advance, Cabbage.

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata* L., 2n = 2x = 18) is one of the most important Cole crops grown under temperate to tropical climate conditions for its head in more than ninety countries throughout the world (Singh *et al.,* 2010) and the leading countries in its cultivation are China, India, Russia, Korea, Japan and the USA. In India, it is next only to cauliflower with acreage and production statistics of 240 thousand ha and 43,000 thousand metric tones, respectively (Atter *et al.,* 2009). It is a rich source of protein comprising all essential amino acids, especially sulphur containing amino acids, minerals such as calcium, iron, magnesium, sodium, potassium, phosphorus and antioxidant and is reported to have anti-carcinogenic properties (Singh *et al.,* 2009). It is also rich source of ascorbic acid, carotene (pro. Vitamin A) and has high fiber content and calcium which reduce the risk of colon cancer (Swarup, 2006). Improvement in any crops depends on the magnitude of genetic variability and the extent of transmission of characters from one generation to the next. The yield and its component characters are polygenic in nature, hence influenced by the environmental factors. Estimation of genetic variability in the germplasm of a particular crop is prerequisite for making any effective breeding programme. Selection of parents to be included in the hybridization programme should be based on genetic distance. Estimation of genetic advance together with heritability would be helpful in assessing the nature of gene action. Genetic coefficient of variation together with heritability estimates gives reliable indication of the amounts of the extent of improvement accepted from selection and further remarked that accepted genetic gain under particular system, which provides true practical information needed by a breeder. Therefore, it is essential to partition the overall variability into its heritable and non-heritable components, which will enhance the precision of selection. Hence and investigation was carried out for estimating genetic variability, heritability and genetic advance among character and its component yield traits in 30 genotypes of cabbage.

MATERIAL AND METHODS

The experimental materials comprised of 30 cabbage genotypes of tropical and subtropical origin belongings to white,

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red and savoy types. Each genotype was planted in a plot having 3.0 x 2.7m area in randomized block design with three replications. There were 25 plants in each plot planted at row and plant spacing of 60 x 45 cm. All the standard package of practices and plant protection measures were timely adopted to raise the crop successfully. Five randomly selected plants from each replication were utilized for recording observations *viz.*, days to maturity, number of non-wrapper leaves, core length (cm), stalk length (cm), equatorial length (cm), leaf length (cm), leaf width (cm), polar length (cm), gross weight (Kg), head weight (Kg) and yield (t/ha) at the Horticultural Research Farm of the Department of Applied Plant Science (Horticulture), BBAU, Lucknow during the winter season of 2006 to 2008. The mean values obtained from 2 years data were used for estimating the analysis of variance (Panse and Sukhatme, 1978). The genotypic and the phenotypic coefficients of variation were calculated by the formula given by (Burton 1951). Heritability in broad sense and genetic advance as per cent of mean were computed by following the methods of Allard (1960) and Johnson *et al.*, (1955), respectively.

RESULTS AND DISCUSSION

Analysis of variance revealed significant differences among the genotypes for all the traits under study (Table 1). The estimates of genetic parameter of variability *viz.*, phenotypic and genotypic coefficient of variation (PCV and GCV) along with heritability in broad sense (h^2) and genetic advance (GA) as percentage of mean for different characters are given in (Table 2). The data revealed that there were significant differences among genotypes for various component traits. High ranges for yield (11.10 - 56.60), leaf width (19.95-46.02) and days to maturity (71.20 - 110.53) were observed. The higher phenotypic coefficient variation than those of genotypic coefficient of variation indicated the predominant role of environment in the expression of traits, which is in confirmation with the result obtained by Jambal *et al.*, (1996) and Singh *et al.*, (2010).

The estimate of PCV and GCV were high for stalk length (PCV=40.42,GCV=39.21), followed by yield (PCV=37.07, GCV=36.87) and core length (PCV=37.43, GCV=36.02), whereas leaf width (PCV=21.51, GCV=21.12), followed by polar length (PCV=16.85, GCV=16.47) and days to maturity (PCV=12.91, GCV=11.50) indicating maximum variability in these characters have ample scope for effective improvement through selection. These results were in broad conformity to those of earlier researchers Swarup and Sharma (1965) and Yadav (1998) in cabbage. Narrow differences between PCV and GCV gave evidence to the genotypes that the variability existing in them was mainly due to their genetic make up.

Heritability is use in predicting the expected progress to be achieved through selection (Burton and De Vane, 1953; Johnson *et al.*, 1955). In the present study, the highest heritability was noticed for the yield (98.90%) followed by leaf length (97.30%), equatorial length (97.10%) and leaf width

Table 1 : Analysis of variance for different characters in cabbage genotypes.

Source of Variance	D.F.	Days of maturity	Non wrapper leaves	Core length (cm)	Stalk length (cm)	Leaf width (cm)	Leaf length (cm)	Equatorial length (cm)	Polar length (cm)	Gross weight (Kg)	Head weight (Kg)	Yield (t/ha)
		Characters										
Replication	2	65.38	4.59	1.43	0.258	2.25	1.52	0.500	1.26	0.912	0.410	1.031
Treatment	29	324.20**	41.39**	25.48**	10.63**	96.03**	98.78**	69.63**	31.47**	0.86**	0.44**	250.57**
Error	58	25.98	1.67	0.66	0.22	1.18	0.93	0.68	0.49	0.075	0.020	0.93

*,**Significant at 5% and 1% level, respectively.

Table 2 : Estimates of genetic constants for different morphological and yield characters in some cabbage genotypes.

S. No.	Character	Range		Mean	Coefficient of Variation		Heritability (%)	Genetic advance	Genetic gain (%)
		Min.	Max.		Phenotypic	Genotypic			
1.	Days of maturity	71.28	110.53	86.96	12.91	11.50	79.40	18.36	21.10
2.	No. of non-wrapper leaves	5.40	23.70	15.16	25.00	24.25	88.90	7.14	47.09
3.	Core length (cm)	2.31	13.85	7.95	37.43	36.02	92.60	5.62	70.69
4.	Stalk length (cm)	2.36	9.46	4.78	40.42	39.21	94.10	3.75	78.45
5.	Leaf width (cm)	14.35	42.75	26.68	21.51	21.12	96.40	11.40	42.73
6.	Leaf length (cm)	15.95	46.02	25.49	22.78	22.47	97.30	11.64	45.66
7.	Equatorial length (cm)	12.10	31.10	20.05	24.19	23.84	97.10	9.71	48.42
8.	Polar length (cm)	12.43	27.40	19.60	16.85	16.47	95.50	6.50	33.16
9.	Gross weight (Kg)	1.20	3.55	2.10	28.28	25.03	78.60	0.96	45.71
10.	Head weight (Kg)	0.49	2.33	1.24	32.23	30.13	87.40	0.72	58.06
11.	Yield (t/ha)	11.10	56.60	24.78	37.07	36.87	98.90	18.72	75.54

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(96.40%). All these traits exhihited more than 90% heritability (Table 2). Number of non-wrapper leaves and head weight showed moderate heritability estimates. Gross weight (78.60) and days to maturity (79.40) showed low heritability estimates. High heritability in broad sense indicated that large proportion of phenotypic variance was attributable to the genotypic variance and that these character differences among the genotypes were real and showed that the above mentioned traits with high heritability values were less influenced by the environment. High heritability for yield and leaf length has been estimated earlier by Yadav (1998) in cabbage.

Johnson *et al.,* (1955) stressed that for estimating the real effects of selection, heritability alone is not sufficient and genetic advance along with heritability is more useful. High genetic advance was observed for yield (18.72), whereas as it was low for core length (15.62), stalk length (3.75), polar length (6.50), gross weight (0.96) and head weight (0.72), which is in broad conformity with the finding of earlier researchers (Swarup and Sharma, 1965 and Lal and Solanki, 1975). Moderate to high genetic advance for days to maturity (Atter *et al.,* 2009), leaf width, leaf length, equatorial length, number of non-wrapper leaves and polar length reported by Joshi (1998) and Lal and Solanki (1975), which was slightly variance with the present findings. High heritability along with moderate genetic advance was observed for leaf length (11.64), leaf width (11.40) and equatorial length (9.71), these findings were at variance with those of Joshi (1998), who reported high heritability associated with low genetic advance. High heritability along with moderate genetic advance for these traits may be attributed to the additive gene action linked with non-additive effects and these may be improved through selection followed by hybridization.

However, high heritability associated with high genetic advance was observed for yield in conformity with the findings of Joshi (1998). High heritability coupled with high genetic advances in yield indicates that this trait is likely to respond better to selection. Stalk length along with core length had moderate heritability associated with low genetic advance and these findings were variance with those of Singh *et al.,* (2002), who reported high heritability associated with moderate advance.

Moderate heritability with low genetic advance was observed for head weight (0.72) which is in contrast to the findings of Swarup and Sharma (1965) who reported low heritability coupled with low genetic advance for this trait. Low heritability in combination with low genetic advance was observed for gross weight (0.96), which varies with the findings of Swarup and Sharma (1965), who reported high heritability with high genetic advance for this trait. Characters with low heritability coupled with low genetic advance are more under the influence of non-additive gene action and environment and do not respond to selection.

High genetic gain (expressed as per cent of mean) was recorded (78.45) followed by yield (75.54), core stalk length (70.69) and head weight (58.06). While the moderate genetic advance of (48.42), number of non-equatorial length wrapped leaves (47.09) gross weight (45.71), leaf length (45.66) and leaf width (42.73), these results are in accordance with the findings of Singh *et al.,* (2002) and Swarup and Sharma (1965).

Thus from the present investigation it may be concluded that characters *viz*; for gross weight, stalk length, leaf length and head weight will be effective to bring rapid improvement in yield and yield attributing traits.

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Mutagens Induced Chlorophyll Mutations in *Vigna radiata* (L.) Wilczek.

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The chlorophyll mutation frequency, effectiveness and efficiency of Ethyl-Methane Sulphenate (EMS), Sodium Azide (SA) and Hydrazine Hydrate (HZ) was evaluated in mungbean variety Asha in M_2 Generation. EMS was found most effective and efficient in inducing chlorophyll mutation frequency. The spectrum of chlorophyll mutations consisted of Albina, Xantha, Chlorina and Viridis, Chlorina type being predominant.

Keywords : Chemical mutagens, Chlorophyll mutations, Effectiveness and Efficiency.

INTRODUCTION

Induced mutagenesis has now been accepted as a significant tool to break through the limitations of variability and to create variability in a short period of time. The possibility offered by mutagenic agents to induce new genetic variations is, therefore of extreme interest. It might in many cases be the only answer to problems posed upon the practical aspects to breeder. A mutation event is indeed very important even when if it has a small effect for a specific morphological or physiological character, because it changes the balance established by natural selection in co-adopted blocks of genes and it, therefore offers new situations for natural or artificial selection.

Chlorophyll mutations although not useful for plant breeding purpose, but it may be used to assess the potency of mutagens. In the present investigation, chlorophyll mutation frequency, effectiveness and efficiency of the mutagens was estimated in the mungbean variety Asha using EMS, SA and HZ.

MATERIAL AND METHODS

The chlorophyll mutations were scored when seedlings were 8-15 days old in M_2 generation. For raising M_2 Generation, 50 healthy seeds of variety Asha from each normal looking M_1 plant of all different treatments with their respective control were planted in the plant progeny rows. The different treatments and controls comprised 30-progenies. The spacing was maintained at 30 cm (Plant to Plant in a row) and 60 cm (between the rows) in the field. Three replications were maintained in each treatment as well as in control.

The chlorophyll mutations were identified and classified as per Gustafsson (1940)'s classification. Formulae suggested by Konzak *et al.* (1965) were used to evaluate the mutagenic effectiveness and efficiency of the mutagens used.

OBSERVATION

Frequency and spectrum of chlorophyll mutations : Chlorophyll mutations were recorded at the seedling stage in the mutagen treated population (Table-1). The frequency of chlorophyll mutants types were observed highest in chlorina followed by Viridis, Xantha, Maculta, Virescent and Albina. In both the chlorophyll mutation frequency increased with the increase in the concentration of mutagens. The EMS induced higher Mutagenic frequency followed by HZ and SA.

Effectiveness and Efficiency : Effectiveness decreases with increase in dose of the mutagens in variety Asha (Table-2). Lower concentrations were most effective in all the mutagenic treatments when compared to higher concentration. The order of mutagen based upon effectiveness was HZ, followed by SA and EMS (Table-2).

In the present study, the mutagenic efficiency, worked out on the basis of seedling injury (MP/I), Pollen Sterility (MP/S) and Meiotic aberrations (MP/MC), showed a decline with increasing concentrations of mutagens in variety Asha of Mungbean. The order of mutagenic efficiency on the basis of seedling injury was HZ > EMS > SA and pollen sterility was EMS > HZ > SA. The order of efficiency on meiotic aberrations (Mp/Me) basis was highest in EMS treated populations followed by HZ. SA treatments did not induce meiotic aberrations.

DISCUSSION

The frequency of chlorophyll mutations increased with the increasing concentrations of the mutagens similar observation were made by Subramanian (1980), Khan *et al* (1998). Chlorophyll mutations may result from alteration in the DNA in the chloroplasts and EMS is known to react preferentially with guanine Freese (1963). With regard to the effectiveness and efficiency, EMS was more effective as well as efficient than SA and HZ. The High efficiency of EMS over

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Table 1 : Frequency and Spectrum of Chlorophyll mutants in M_2 generation of mungbean var. Asha

Treatment	No. of M_1 Plant progenies	No. of Plant progenies Segregating in M_2	% Mutated Plant Progenies (Mp)	Chlorophyll mutant types							
				Albina	Xantha	Chlorina	Maculata	Viridis	Virescent	Total	Frequency (%)
Control	28	--	--	--	--	--	--	--	--	--	--
0.1% EMS	30	12	40.00	--	4	8	2	4	2	20	3.32
0.2% EMS	30	14	46.66	2	6	10	2	6	2	28	4.70
0.3% EMS	31	16	51.66	2	5	12	4	5	3	31	5.48
0.4% EMS	30	19	63.33	3	7	15	5	6	4	40	7.85
0.01% SA	30	8	26.66	--	1	5	--	2	--	8	1.34
0.02% SA	30	9	30.00	--	2	4	--	2	2	10	1.70
0.03% SA	31	11	35.48	--	2	5	2	4	--	13	2.30
0.04% SA	28	10	35.71	1	4	6	3	5	2	21	3.96
0.01% HZ	30	9	30.00	--	3	9	--	3	--	15	2.45
0.02% HZ	30	11	36.66	--	3	10	1	4	--	18	2.99
0.03% HZ	32	13	40.62	2	4	12	2	4	2	26	4.42
0.04% HZ	29	14	48.27	2	5	12	4	5	4	32	6.15

Table 2 : Effectiveness and Efficiency of different mutagens in M_2 generation of mungbean var. Asha

Treatment	% Seeding Injury (I)	% Pollen Sterility (S)	% Meiotic Aberrations (Me)	% Mutated Plant Progenies (Mp)	Mutagenic Effectiveness (Mp/t.c.)	Mutagenic Efficiency		
						MP/I	Mp/S	Mp/Me
0.1% EMS	2.58	10.69	2.93	40.00	66.66	15.50	3.74	13.65
0.2% EMS	6.41	18.61	5.68	46.66	38.88	7.27	2.50	8.21
0.3% EMS	13.69	27.46	9.89	51.66	28.70	3.77	1.88	5.22
0.4% EMS	23.27	33.10	16.01	63.33	26.38	2.72	1.91	3.95
0.01% SA	3.83	09.25	--	26.66	444.33	6.96	2.88	--
0.02% SA	13.40	15.97	--	30.00	250.00	2.23	1.87	--
0.03% SA	24.80	23.09	--	35.48	197.011	1.43	1.53	--
0.04% SA	33.62	28.62	--	35.71	148.79	1.06	1.24	--
0.01% HZ	1.62	10.05	2.40	30.00	555.50	18.51	2.98	12.50
0.02% HZ	4.98	18.41	4.95	36.66	361.08	7.36	1.99	7.40
0.03% HZ	9.86	26.30	7.92	40.62	260.38	4.11	1.54	5.12
0.04% HZ	21.26	32.20	12.56	48.27	201.12	2.27	1.49	3.84

physical mutagens was also advocated by Goud (1967) and Uhlik (1972). The high efficiency at lower doses may be attributed to less toxic effects of low doses. Dose dependent increases in meiotic abnormalities were with EMS and HZ treatment. Similar observations were also reported by Ignacimuthu and Babu 1989, Reddy *et al.* 1991.

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Antifungal Activity of the Essential Oil of *Eucalyptus laveopinea* Against Skin Infective Fungi

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We investigated here, the essential oil of *Eucalyptus laveopinea, in vitro* showed broad range fungicidal activity at 2.0 µl/ml concentration against human pathogenic fungi, *Epidermophyton floccosum, Microsporum audouinii, M. nanum, M. canis, M. gypseum, T. mentagrophytes, T. rubrum, T. violaceum* and *T. tonsurance.* The oil has potency against heavy doses of inoculum at 2.0 µl/ml and its toxicity didn't expire even upto 72 month of storage. Moreover, oil didn't exhibit any adverse effects on mammalian skin upto 5% concentration. Thus, in future the oil in the form of ointment can be used against dermatophytosis , had long shelf life (72 month) and absence of any adverse side effects.

Keywords : Antifungal, Essential oil and Human pathogenic fungi.

INTRODUCTION

Superficial fungal infections (dermatophytoses) occur more frequently than subcutaneous and systemic mycoses and remain a therapeutic problem in tropical and subtropical countries[1] despite the availability of a number of antifungal ointments, paints, lotion and powders. Sources of these agents are largely nonrenewable petro-products that are nonbiodegradable and cause adverse effects and residual toxicity[2]. Thus, the meaningful search of new treatment with better and cheaper substitutes-plant resources are natural choice.

Naturally occurring fungitoxicants described to date are mostly biodegradable and are devoid of side effects[4] compared with commercially available fungitoxicants. Recently, essential oils of higher plant origin have been shown to be effective source of chemotherapeutic agents without undesirable side effects and with strong fungicidal activity[5]. These findings prompted us to explore other plant products (essential oil), which could be exploited as effective fungitoxicant. We report here results of our investigation, the essential oil of *Eucalyptus laveopinea* Baker, a member of the family Myrtaceae, as an effective antifungal against, *Epidermophyton floccosum* (Hartz) Langeron et Milochevitch, *Microsporum audouinii* Gurby, *M. canis* Bodin, *M. nanum* Fuentes *M. gypseum* (Bodin) Guiart et Grigoraki, *T. mentagrophytes* (Robin) Blanchard, *T. rubrum* (Castellani) Sabouraud, *T. tonsurans* Malmstem, *T. violaceum* (Bodin).

MATERIAL AND METHODS

The essential oil was extracted from the fresh leaves of *E. laveopinea* by hydro-distillation using Clevenger's apparatus[10].

In vitro studies, the minimum inhibitory concentrations (MICs) of the oil against dermatophytes were determined following the poisoned food technique (PFT) of Grover and Moore[11] with slight modification[8]. The requisite quantity of the oil samples were mixed in acetone (2% of the required quantity of the medium) and then added in pre-sterilized sabouraud dextrose agar (SDA) medium (pH- 5.6). In control sets, sterilized water (in place of the oil) and acetone were used in the medium in appropriate amounts. Mycelial discs of 5-mm diameter, cut out from the periphery of 7-day old cultures of the test dermatophytes. Pathogens were aseptically inoculated upside down on the agar surface of the medium. Inoculated petri plates were incubated at 27 ± 1°C and the observations were recorded on seventh day. Percentage of mycelial growth inhibition (MGI) was calculated as per formula.

MGI= (dc–dt) x 100 / dc

where, dc = fungal colony diameter in control sets, dt = fungal colony diameter in treatment sets

To find out the minimum fungistatic/fungicidal concentrations of the oil at minimum inhibitory concentrations (MICs) were ascertained by the method of Garber and Houston[12]. This was done by, reinoculated the inhibited fungal discs at MICs on SDA medium. Observations were recorded

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Table 1 : Minimum inhibitory concentrations of the oil of *Eucalyptus laveopenia* against test pathogens.

Concentration (µl/ml)	Mycelial Growth Inhibition (MGI %)								
	Ef	*Ma*	*Mc*	*Mn*	*Mg*	*Tm*	*Tr*	*Tt*	*Tv*
3.0	100^c	100^c	100^c	100^c	100^c	100^c	100^c	100^c	100^c
2.0	100^c	100^c	100^c	100^c	100^c	100^c	100^c	100^c	100^c
1.0	100^s	100^s	100^s	100^s	100^s	100^s	100^s	100^s	100^s
0.8	100^s	100^s	100^s	100^s	100^s	100^s	100^s	100^s	100^s
0.6	40.0	36.0	36.0	31.1	43.0	33.9	55.5	34.0	56.0
0.4	30.5	26.2	65.1	55.2	69.1	70.0	73.1	69.0	69.2

Ef, *Epidermophyton floccosum*; Ma, *Microsporum audouinii*; Mc, *M. canis*; Mn, *M. nanum*; Mg, *M. gypseum*; Tm, *Trichophyton mentagrophytes*; Tr, *T. rubrum*; Tt, *T. tonsurance*; Tv, *T. violaceum* ; 100^s, 100% inhibition with fungistatic action ; 100^c, 100% inhibition with fungicidal action.

after 7-day incubation. Fungal growth on seventh day indicated fungistatic nature while absence of fungal growth denoted fungicidal action of the oil.

The effect of inoculum density (increased progressively up to 30 discs in multiple of 5 and each of 5-mm diameter) of the test pathogens on MICs of the oil was determined following the procedure outlined by Dikshit and Dixit[13]. Effect of some physical factor *viz.*, temperature and expiry of toxicity during storage of the oil was evaluated according to Shahi *et al.* [9]. Five lots of oil were kept in small vials, each containing 5-ml of oil; these were exposed to different temperatures, 40, 60, and 80 °C in incubator for an hour. Antifungal activity then tested at MICs by the usual poisoned food technique[11]. Expiry of toxicity of the oil was determined by storing the oil at room temperature and antifungal activity was tested at MICs by the regular intervals of 60 days up to 72 months following the poisoned food technique[11]. All the experiments were repeated twice and each contained five replicates and the data presented here are based on their mean values.

RESULTS

The oil showed broad range of antifungal activity at 2.0 µl/ml against *Epidermophyton floccosum, Microsporum audouinii, M. canis, M. nanum, M. gypseum, T.mentagrophytes, T. rubrum, T. violaceum* and *T. tonsurance* with fungicidal nature (Table 1). The oil inhibited heavy doses of inocula exhibited 100% mycelial growth at its respective fungicidal concentration.

DISCUSSION

Antifungal compound should be tested under both *in vitro* and *in vivo* conditions in order to prove its potential as promising fungitoxicant for the control of the disease. Since, detailed *in vitro* studies on the essential oil of *E. laveopinea* indicate their potentiality as ideal antifungal compound against dermatophyte, these were further subjected to *in vivo* investigation so as to confirm their efficacy as therapeutant for the control of dermatophytoses. The oil did not show any irritant activity at 5% concentration but the ointment were prepared much less concentration *i.e.* 1% concentration. Beside, the *Eucalyptus* oils which is used in various pharmaceuticals preparations mentioned in the Merck Index lists as an indicative of harmless to human health, can easily be obtained from renewable natural resources.

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Evaluation of Antifungal Potency of Certain Indian Drug Plants *in vitro*

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Ten crude aqueous extracts from different parts of five plant species *i.e., Azadirachta indica, Adhatoda vasica, Caltropis procera, Ficus religiosa* and *Ephedra foliata* were evaluated for their therapeutic potential as antifungal agents against three human pathogenic (*Candida albicans, Trichophyton mentagraphytes* and *Microsporum gypseum*) and two plant pathogenic (*Colletotrichum* sp and *Fusarium* sp) fungi. All the plant extracts exhibited profound antifungal activity against most of the test fungi used in the present study. Antifungal activity of *Azadirachta indica* (bark and leaves) and *Ficus religiosa* (bark) at 100% concentration against *T. mentagrophytes* and *C. albicans* was found maximum and was to be equal to that of antifungal agent Candid-B, a well known drug recommended and used in several mycoses in the modern medicine system. A positive co-relation was found between the drug concentration and antifungal potency. Declined trend of antifungal potency was increased with raised dilutions of extract. The present findings opens the hope for alternative avenues as substitute of modern drugs (synthetic drug and antibiotic) for the management of human diseases caused by above test fungal organism. Findings further encourage to use these plant based substances other than these dermatphytes and plant pathogens and also an urgent need to screen the other drug plants for their antifungal potency against the above test dermatophytes and other human fungi in order to make a proper and low cost mycoses management for third world countries.

Keywords : Antifungal activity, Plant extracts, *Ficus religiosa, Azadirachta indica, Candida albicans, Trichophyton mentagrophytes.*

INTRODUCTION

Fungi occur ubiquitously and are well adapted to use a wide range of substrates as their carbon, nitrogen and energy source. The growth of many fungi especially difficult to control because of their ability to metabolize many substances (Quiroga *et al.*, 2001). These organisms can cause serious diseases in plants, animals and humans. Human infections particularly those involving the skin and mucous membranes, are increasing at an alarming rate, especially in tropical and sub-tropical developing countries, with dermatophytes and *Candida* sp. being the most common pathogens. This increase is directly related to the growing number of immunosupressed and immunocompromised patients, who frequently develop opportunistic systemic and superficial mycoses such as candidiasis, cryptococcosis and filamentous fungal infections (Li *et al.*, 1995; Groll *et al.*, 1996; Portillo *et al.*, 2001). It appears more severe and common in the patient suffering with HIV infection and getting declined immunity with progression of AIDS. Human mycoses are not always successfully treated because antimycotic drugs are limited by a number of factors such as low potency, poor solubility, emergence of resistant strains and drug toxicity (Formtling and Rahway, 1987). It is therefore essential to search for more effective and less toxic antifungal agents without problems of drug resistant (MeCutcheon *et al.*, 1994; Zacchino *et al.*, 1999; Fernner *et al.*, 2005). Traditional medicine has been found a potential source of new antibacterial and antifungals even against MDR pathogens. Belsem *et al.*, (2009) reported that *Citrullus coloeynthis* schrad, (Cucurbitaceae) has been used as folk medicines against dermatological, gyaenoculogical and pulmonary infection in Southern Tunisia. They studied the antifungal potency of different parts of this plant against three species of Candida along with certain bacterial species and found a strong inhibitor in vitro. Saratha and Subramanian (2010) studied the antifungal activity of latex of *Calotropis gigantia* against *Trichophyton mentagraphytes, Candida albicans*, along with other fungal species. Balasubramanian *et al.*, (2010) studied the antifungal activity of certain medicinal plants against *C.albicans*. Suleiman *et al.*, (2010) studied the antifungal efficacy of certain trees against five fungal species including several human pathogens.

In diseases of microbial origin, the plants function as a result of antimicrobial activity against the causative agents (Onyeagb *et al.*, 2004). Drawing attention to their potential as additional sources of antibiotic is very important (Larhsini *et al.*, 2001). Application of the plants as whole or their different parts as a single drug plant or with combination of other plants

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have been made in the treatment of large number of skin diseases. Some important plants capable to treat various skin diseases are mentioned as bark of *Azadirachta indica* and leaves of *Casia fistula*. Parts of leaves of *Casia fistula* has been described as potent for treatment of Eczema and other skin disease.

Inspite of having sufficient information regarding potency of the drug plant in the management of skin disease such effort has not been made earlier in this direction. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compound isolated from natural products (Nair *et al.*, 2005). In the light of the above, present work has been carried out to evaluate the potency of aqueous crude extracts from five plants belonging to different families against three human pathogenic fungi and two plant pathogenic fungi.

MATERIAL AND METHODS

Test Plant Material : The test plant materials (*i.e., Azadirachta indica, Adhatoda vasica, Calotropis procera, Ficus religiosa* and *Ephedra foliata*) were collected from various localities of district Kurukshetra (Haryana). Details of the test plants and their ethnomedicinal uses and plant parts used to obtain the extracts have been presented in Table-1. Three human pathogenic fungi *i.e., Candida albicans, Trichophyton mentagrophytes* and *Microsporum gypseum* were procured from Prof. (Dr.) S.S. Thukral, Head, Department of Microbiology, Sardar Vallabhbhai Patel Chest Institute, University of Delhi, Delhi - 110067. The two plant pathogens *i.e., Fusarium* sp., *Colletotrichum* sp. were isolated from the infected Guava fruits. Fungal test organisms were grown and sub-cultured fortnightly on Potato dextrose agar at 26ºC.

Synthetic compound : The antimycotic agent Candid-B (Glenmark Pharmaceuticals Ltd.) was included in the present investigation as positive control. The candid - B was containing clotrimazol I.P. 1% w/v.

Preparation of extracts : The freshly collected plant parts were thoroughly washed with sterile distilled water 2-3 times and surface sterilization was done with 90% alcohol (Gehlot and Bohra, 1998). The material was dried in an oven at 50 ºC for 48 hrs. The dried plant material was grinded to a fine powder. The powdered materials were kept in separate sterile flasks and stored in a refrigerator at 4 ºC until use (Lin *et al.*,1999).

For aqueous extraction, 250 g powdered plant material was mixed with 1000ml sterilized distilled water (25% w/v, excess water was also added wherever required) and macerated in a pestle and mortar. The mixture was kept undisturbed at ambient temperature (29 ± 1ºC) for 24 hrs in a sterile flask covered with a plate to avoid evaporation followed by maceration in a pestle and mortar. The mixture was then subjected to filtration through sterilized double layered muslin cloth and sterile filter paper (Whatmann no. 1). After filtration, the extract was allowed to evaporate at the same temperature to get 250ml solution. Final concentration of the extract was 1.0 g/ml (w/v, based on the dry weight of the plant material) or in other words, 1.0 ml of extract solution represented 1.0 g of powdered material (Chen *et al.*,1987; Barreto *et al.*, 2002). The resulting solution obtained was designated as 100% extract. The plant extracts were immediately used after preparation for performing antimicrobial bioassays without any further storage. Three dilutions (75%, 50%, and 25%) of each plant extract were prepared by using sterile distilled water and 100% concentrated extract was used as such without addition of sterile distilled water. Antifungal potency of each concentration of all crude extract was evaluated.

Antifungal potency assay : Antifungal activity of aqueous extracts and synthetic compound was determined by Poisoned Food technique (Nene, 1971) that involves the measurement of radial growth of a test fungus in the presence and absence of plant extracts. Six days old fungal cultures were used as inocula for testing antifungal activity of the plant extracts. 10 ml undiluted (100%) plant extract was added to 90 ml molten (45 - 50ºC) PDA and mixed well before use. The same process of addition was repeated with the remaining diluted (75%, 50%, and 25%) plant extracts. For control, 10 ml sterilized distilled water was mixed with molten 90 ml (45-50 ºC) PDA. The PDA medium containing plant extract was poured into 90 mm Petri dishes and agar was allowed to set. Similarly, PDA medium containing control (sterile distilled water) was also poured into 90 mm Petri dishes and allowed to solidify. Now, PDA plates having plant extracts were inoculated at the centre with the mycelia disc (6.0 mm diameter) of the test fungus. The control PDA plates were also inoculated in the same manner. Both test and control PDA plates were incubated at 26 ± 1ºC in a BOD incubator for six days. After incubation period, the PDA plates were observed for the presence or absence of mycelia growth of the test fungi. The growth of the fungal colonies was obtained by measuring the diameter of the colony (in mm) across the two lines drawn at right angles to each other. The mean of the two measurements was used to calculate the diameter of the colony (Barreto *et al.*, 2002). The growth inhibition (%) of the test fungi was calculated by applying formula (Chilpa *et al.*,1997).

Growth inhibition (%) =
mycelial growth in control - mycelial growth in plant extract × 100 / mycelial growth in control

RESULTS AND DISCUSSION

Plants capable of sensing the presence of potential phytopathogen (fungi, bacteria and viruses) and mounting defense mechanisms. Plant can produce antifungal compounds to protect themselves from biotic attract that could be essential for fungi infection resistance (Wojtaszek, 1997) keeping in view of the potency of the drug plant to synthesize secondary metabolites with in them to protect from the microbial pathogens including fungi, efforts have been made to find out the potency of the crude drug extract against dermatophytes and certain plant pathogenic fungi. Since several human pathogenic fungi are associated with different mycoses (local & systematic) therefore the investigation becomes more significant. Albeit plants have been applied locally for the skin infection since ancient time but study becomes an imperative

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in connection of modern concept *i.e.*, causal organization theory. In the present study, ten aqueous crude extract of five plant species were evaluated for their antifungal activity against four human pathogenic fungi (dermatophytes) and one plant pathogenic fungi. Results obtained in the present investigation have been presented in Table 2.

For most of the extract, the activity was found to be concentration dependent *i.e.*, reduction or complete loss of antifungal activity was noticed with the dilution of plant extracts. The complete loss of antifungal activity was noticed with the dilution of plant extracts. The complete loss of antifungal activity with dilution was noticed in the leaf extract of *A.indica*, where diluted 25% extract showed no growth inhibition against *M. gypseum* (Table 2). Significant difference among antifungal activity of undiluted and diluted 25% extract was observed.

Maximum antifungal activity against *C.albicans* was observed in the aqueous bark extract of *A. indica*, that showed complete growth inhibition (100%) till the lowest concentration of extract. Okemo *et al.*, (2001) had reported that death of complete population of *C.albicans* was found in the bark extract of *A.indica* at a concentration of 8 mg/ml in 24 hrs. A similar antifungal potency against *C.albicans* has been recorded from *Dodonaea viscose* extract by Patel and Coogan (2008). They found that all the strains were killed with in 30 second. Balasubramanian *et al.*, (2010) studied antifungal activity of five drug plants against *C.albicans* which is the second most important cause of vaginitis *i.e.*, vulvovaginal candidiasis (VVC). Maximum inhibition was found in extract of *Acalypha indica* among all tested plants.

Candida albicans and *T. mentegrophytes* were sensitive to most of the plant extracts, but both test fungi were also found to be resistant to the extract of leaves of *A. indica* and aerial parts of *E. foliata*. The remaining three test fungi *i.e. M.gypseum, Colletotrichum* sp. and *Fusarium* sp. were sensitive to all the plant extracts. It was noticed that some plant extracts (*A.indica*, leaves and bark; *F. religiosa*, bark) showed complete growth inhibition (100%) against *C.albicans* and *T.mentagrophytes*, but none of the plant extract could produce complete growth inhibition (100%) against *M.gypseum, Colletotrichum* sp., and *Fusarium* sp. (Table 2). The undiluted bark extract of *F.religiosa* produced 52.38% growth inhibition against *C.albicans*. The remaining extracts were completely unable to produce even 50% growth inhibition of *C.albicans* (Table 2).

Complete growth inhibition (100%) against *T. mentagrophytes* was found in the extracts of *A. indica* (leaves and bark) and *F. religiosa* (bark) till the lowest concentration. On the other hand, the percent growth inhibition against *T.mentagrophytes* by the extracts of *A.vasica* (leaves and inflorescence) and *C.procera* (flowers) was ranging between 50% and 59.09%. The growth inhibition of the extracts of *A.vasica* (shoots) and *C.procera* (leaves and shoots) against *T.mentagrophytes* was found less than 50% (Table 2).

Highest growth inhibition (70%) against *M.gypseum* was demonstrated by the undiluted bark aqueous extract of *F. religiosa*. Other extracts showing more than 50% growth inhibition against *M. gypseum* were from *A.indica* (bark), *C.procera* (flowers) and *A. vasica* (leaves, inflorescence and shoots). The growth inhibition of *M. gypseum* by the extracts of *A.indica* (leaves), *E.foliata* (aerial parts) and *C.procera* (leaves and shoots) was found to be below 50% (table 2).

Maximum growth inhibition against *Colletotrichum* sp. was observed in the undiluted extracts of *A.indica* bark (91.17%) followed by *F. religiosa* bark (72.50%). The extracts of *A.vasica* also demonstrated 50% or more growth inhibition against the plant pathogenic fungus (Table 2). After bark extracts, the most promising extract against *Colletotrichum* sp was from *A.vasica* inflorescence, that produced 64.51% growth inhibition against the test fungus. The growth inhibition against *Colletotrichum* sp. by the extract *A.indica* (leaves), *E.foliata* (aerial parts) and *C.procera* (leaves, shoot and flowers) extract was found to be less than 50% (Table 2).

Bark extract of *A.indica* and *F.religiosa* were found to be most promising against plant pathogenic *Fusarium* sp. with 82.89% and 78.18% growth inhibition respectively (Table 2). The remaining plant extracts were completely unable to produce even 50% growth inhibition against the *Fusarium* sp.

Of various plant parts collected and tested, which included the bark, leaves, shoots, inflorescence, flowers and whole aerial parts, the bark had significant higher antifungal activity. This indicates that bark being the outermost protective part of the tree is well defended against the fungal attack. However, the antifungal activity of *A.indica* (leaf) against *T.mentagrophytes* is an exception to this observation. Aqueous extract of *A.indica* leaf produced complete growth inhibition against *T.mentagrophytes*, which was equally comparable to that of *A.indica* bark, *F.religiosa* bark and candid-B. because leaves are more sustainable resource than bark and can be used without any detrimental effect on plant. Therefore, this makes leaf extract more promising than the bark for further antifungal studies against *T.mentagrophytes*. Moreover, the difference among antifungal activity of *F.religiosa* bark (72.50%) and *A.vasica* inflorescence (64.51% growth inhibition) against *Colletrotrichum* sp. was also found to be small, making inflorescence better choice than bark against this plant pathogenic fungus, that represents threat to the health of humans ingesting contaminated feeds and foods.

The antifungal potential of most of the plant extracts has also turned out to be quite weak in comparison with that of candid B. But the results are interesting and can not be under estimated because the present study deals with crude phytochemical extracts and not a pure product. Hence, extract real concentration of the active principles of extracts are likely to be lower than those present in the standard antifungal agent.

The antifungal agent candid-B showed complete growth inhibition (100%) against all the human and plant pathogenic fungi tested (data not shown). The results indicate that the antifungal activity of *A.indica* (leaf and bark) and *F.religiosa* (bark) against *T.mentagrophytes*, a parasite of hair causing dermatophytic infections, was equal to that of antifungal agent

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candid-B. Similarly growth inhibitory activity of *F.religiosa* (bark) against *C.albicans* that causes superficial infections of the skin and mucous membranes has also been found equal to that of candid-B. The infections of *C.albicans* are more pronounced in the patients suffering from HIV infection. A couple of decades ago, *Candida albicans* was commonly regarded as little more than culture contaminant; however because of developed antimicrobial resistance in less than 2 decades, this organism has become a major human pathogen (Desta, 1993; Goff *et al.*, 1995; Nolte *et al.*,1997; Kieren *et al.*, 1998; Michael and Pharm, 2001; Bonjar, 2004). The mortality rate reported for patients with *C.albicans* is the highest among all fungal pathogens (Crump and Collington, 2000). The important observation is that these crude phytochemical extracts are highly diluted and even their lowest concentration produced complete growth inhibition against *C.albicans* and *T. mentagrophytes*, leading to the distinct possibility that these extracts may be applied clinically for dermatophyte infections.

Most of the plant extracts were considered to be broad antimycotic because they demonstrated activity against the panel of both human and plant pathogenic fungi. Therefore, they may be considered for the development of more cheap extracts and elucidation of the active components responsible for the activities along with *in vitro* studies of the phytochemicals is recommended before any use of these bioactive compounds in medicinal or food value in order to determine their effects in the whole organism systems including toxicity and effect on the normal microbial flora of humans. The understanding of the inhibitory mechanism would provide better directions towards the development of efficient production and application of technologies associated with biofungicide plant materials (Quiroga *et al.*, 2001).

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Table 1 : Ethnobotanical data of the studied plants[a].

S. No.	Family	Species	Plant Part	Vernacular Names	Claimed Therapeutic Uses
1.	Meliaceae	*Azadirachta indica*	Leaves and Bark	Neem	Bark used in skin troubles, leaves considered antiseptic, applied to boils in the from of poultice, decoction given for ulcers and eczema.
2.	Acanthaceae	*Adhatoda vasica* Nees.	Leaves, shoots and inflorescence	Arusha	Fresh or dried leaves constitute the drug Vasaka, used in bronchial troubles, leaf juice also used in diarrhea, dysentery and glandular tumors, powdered leaves are also used in skin infections.
3.	Asclepiadaceae	*Calotropis procera* (Aiton) W.T. Aiton	Leaves, shoots and flowers	Aak	Root bark used for leprosy
4.	Euphorbiacea	*Ephedra foliata* Boiss. and Kotschy ex Boiss.	Aerial parts	None	none
5.	Moraceae	*Ficus religiosa* Forssk	Bark	Pipal	Infusion used for ulcers and skin troubles.

[a] All the information regarding ethnomedicinal data has been taken from: The Useful Plants of India (Anonymous, 1986)

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Table 2 : Antifungal activity of aqueous extract at different concentrations.

Plant (Plant parts)	Extract Concentration	Growth inhibition percentage[a]				
		Ca	Tm	Mg	Cl	Fu
Azadirachta indica (Leaves)	100%	--[b]	100.00	24.00	42.59	47.61
	75%	--	100.00	20.00	29.62	46.03
	50%	--	100.00	16.00	25.92	42.85
	25%	--	100.00	--	18.51	34.92
Azadirachta indica (Bark)	100%	100.00	100.00	56.52	91.17	82.89
	75%	100.00	100.00	52.17	80.88	82.55
	50%	100.00	100.00	52.17	77.94	81.57
	25%	100.00	100.00	49.27	77.94	80.26
Ficus religiosa (Bark)	100%	52.38	100.00	70.00	72.50	78.18
	75%	47.61	100.00	66.00	67.50	65.45
	50%	42.85	100.00	56.00	42.50	61.81
	25%	38.09	100.00	54.00	35.00	58.18
Ephedra foliate (Aerial parts)	100%	36.00	--	39.70	42.85	61.81
	75%	28.00	--	39.70	30.15	58.18
	50%	24.00	--	23.52	26.98	23.17
	25%	8.00	--	11.76	4.76	19.51
Calotropis procera (Shoots)	100%	28.57	42.85	30.30	28.57	38.82
	75%	23.80	42.85	28.78	21.42	38.82
	50%	23.80	35.71	27.27	21.42	36.47
	25%	4.76	21.42	25.78	14.28	35.29
Calotropis procera (Leaves)	100%	26.66	18.18	36.17	20.00	32.75
	75%	26.66	18.18	29.78	20.00	32.75
	50%	13.33	9.09	29.78	20.00	29.31
	25%	13.33	9.09	25.33	13.33	29.31
Calotropis procera (Flowers)	100%	33.33	59.09	54.00	43.33	40.00
	75%	20.00	54.54	52.00	36.66	30.66
	50%	13.33	50.00	38.00	33.33	30.66
	25%	13.33	45.45	34.00	20.00	26.66
Adhatoda vasica (Shoots)	100%	31.25	47.05	55.76	50.90	44.44
	75%	31.25	35.29	51.92	43.63	37.50
	50%	25.36	29.41	51.92	43.63	30.55
	25%	25.36	23.52	42.30	40.00	30.55
Adhatoda vasica (Leaves)	100%	23.07	50.00	55.55	51.61	45.20
	75%	15.38	50.00	44.44	35.48	39.72
	50%	15.38	43.00	44.44	29.03	34.24
	25%	7.69	43.00	33.33	29.03	31.50
Adhatoda vasica (Inflorescence)	100%	31.25	52.63	55.55	64.51	42.85
	75%	31.25	48.24	46.66	59.67	42.85
	50%	25.00	42.36	37.77	59.67	38.58
	25%	25.00	41.00	37.77	43.54	34.00

[a]Abbreviations used :
Ca-*Candida albicans*; Tm-*Trichophyton mentagrophytes*;
Mg-*Microsporum gypesum*; Cl-*Colletotrichum* sp.; Fu-*Fusarium* sp.
[b](–) Nil growth inhibition.

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Aquatic Macrophytes of Panidihing Bird Sanctuary of Sivasagar District and Their Ethnomedicinal Importance

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Sivasagar district situated in the south bank of mighty Brahamputra river, covers total area of 2668 square kilometer and lies between 94°15' and 95°45' East longitude and 26°45' and 27°15' North latitude. Amongest all biogeographic zones in India the northeast zone is perhaps the richest in communites, species and in endemics. A comprehensive study of the aquatic and semi aquatic macrophytic plant species of Panidihing Bird Sanctuary of Sivasagar, Assam has been made from June, 2008- October, 2010. During the study recorded 20 medicinally important plant species of 15 families, 9 species belonging to eight dicot families and eleven species of seven monocot families have been recorded. Enumeration of plant species established that aquatic macrophytes play an important role in the field of emerging ethnobotany on the district. Local inhabitants of the district used plant species to the treatment of different diseases.

Keywords : Macrophytes, Panidihing Bbird Sanctuary, Ethnomedicinal plants.

INTRODUCTION

Sivasagar district of northeast India endowed with luxuriant vegetation and rich in biodiversity. The district comprised of one sanctuary, six reserve forests and 23 grazing forest area. *Viz.*, Panidihing bird sanctuary, Abhoypur, Sola, Sapekhati, Rangoli, Dili and Galekey reserve forest. Abhoypur reserve forest is the transition area in between Assam- Nagaland and Assam-Arunachal Pradesh. The district comprised of innumerable large and small sized natural wetlands which harbors a rich biodiversity ranging from microscopic planktons to highly evolved angiosperms and mammalian forms of life. These wetlands are the place of habitat of many medicinally and economically important aquatic and semi aquatic flora and fauna. Most of these wetlands are created by the rivers and rivulets by changing their courses and also by natural depression. Frequent flood and erosion also contribute to the formation of these wetlands.

Panidihing and Saragua are two historically important places. Ahom king made capital at Saragua near Panidihing. Dhai Ali an important road linked with history of Assam passes near by. The area is situated about 35 km. East of Sivasagar town and geographically located at 94°40' East longitude and 27°10' North latitude. The whole Bird Sanctuary is surrounded by 3 tributaries of Brahmaputra namely Disang, Demow and Dihing. It shows a heterogeneous flora.

The vegetation of Assam has been studied floristically since long back by several workers as Hooker 1872-1897; Kanjilal *et al.* 1934-1940 ; Bor 1940; Gogoi 1982; Islam 1984, 1990; Borthakur 1993; Gogoi and Islam 2006; Gogoi and Zaman 2008. But limited work has been done in aquatic macrophytes of the district. Therefore an attempt has been made to study the ethnomedicinal aspects of aquatic plants of this area.

MATERIAL AND METHODS

Survey was conducted between June, 2008 and October, 2010 particularly during rainy seasons. It was observed that this region is very rich in aquatic plant diversity moreover huge numbers of migratory birds come here during winter. During the survey specimens were collected and herbarium prepared (Jain and Rao, 1976) and deposited in the laboratory of Kalanchupar research institute. Ethnomedicinal importance of aquatic plant of the sanctuary were gathered through the interaction with the local in habitants with personal interaction perticularly age-old person and tribal people *viz.*, Mising, Deori, Sonowal kacharis and Tai Ahoms. They use several aquatic macrophytes as medicines regularly. Photographs of the plant and local names of the species were recorded during the field study.

OBSERVATION

In the following ethnobotanical enumeration, the plant species are presented in alphabetical order of botanical name, followed by family in perentheses, local name, plant part (s) and local formulations plant part used.

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Acorus calamus L. (Araceae) Boch. Underground rhizome use to treat cough and influenza.

Alternanthera philoxeroides Griseb. (Amaranthaceae) Pani khutora. Young shoots use as vegetables during stomach disorder.

Alpinia allughas (Retz) Rosc. (Zingiberaceae) Tora. Rhizome used to cure nail ring worms.

Calamus rotung L.(Arecaceae). Bet. split stem used to cure nail infection and young shoot used in treatment of thread worm.

Clinogyne dichotoma Salis. (Marantaceae) Patidoi. Rhizome used to treat burn injuries.

Colocasia esculenta Schott. (Araceae).Kochu. Cooked young leaves given to mothers after delivery.

Commelina benghalensis L.(Commelinaceae). Kona simolu. Plant juice applied on eye lid to cure eye lid sore.

Cyperus esculentus L.(Cyperaceae). Sesur. Tubers used as antilithic.

Eclipta prostrata L.(Asteraceae) Keheraj. Whole plant juice used to blacken the hair.

Enhydra fluctuans Lour. (Asteraceae). Helochi sak. Cooked young shoots used to cure stomach disorder.

Ipomoea aquatica Forsk.(Convolvulaceae). Kolmou sak. Cooked young shoots given to anemic persons.

Lasia spinosa (L.) Thw. (Araceae). Chengmora. Young leaves used in piles.

Limnophilla aromatica (L.) Druce. (Scrophulariceae). Am bon. Smeared leaves inhale to sneeze in headache.

Ludwigia octovalvis (Jacq) Raven (Onagraceae). Long bon. Plants used as expectorant and vermifuge.

Nelumbo nucifera Gaertn (Nymphaceae). Podum. Powdered root used against piles, dysentery and diarrhea

Nymphaea lotus (L.) Hook.f. (Nymphaceae).Bhet. Powdered root stock used in stomach problem.

Monochoria hastaefolia Presl. (Pontederiaceae). Bhat meteka. Leaf juice applied to boils.

Polygonum hydropiper L. (Polygonaceae). bihlongoni. Plant use to treat uterine disorder.

Sagittaria saggitifolia L.(Alismataceae). Jathipotia. Powdered leaves used the relief of itch.

Scirpus grossus L.f. (Cyperaceae). Keheru. Tuber used in diarrohea and vomiting.

DISCUSSION

It has been realized from the study that, this particular bird sanctuary of upper Brahamputra valley provide very useful and rare data on ethnobotany. The intensive study may revealed a treasure of traditional knowledge acquired by the tribal from their forefather who were totally dependent on forest plant wealth. There is no record on the occurrence and distribution of aquatic, semi-aquatic and marshlands plants of the district. Because of shrinking habitats of aquatic plants due to conversion of low lands for the human settlements, road constructions, drilling operation by ONGC Limited and the extension activity of small tea gardens. 20 aquatic macrophytes have been observed which are used as medicine by the local inhabitants of the district.

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Population Flux of the Medicinal Shrub, *Gaultheria fragrantissima* Wallich in Nilgiris, the Western Ghats, India

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Observations were made on population flux of four ecological variants of *Gaultheria fragrantissima viz*, ovate, lanceolate, elliptic - lanceolate and oblanceolate leaf forms in four major shola forests of Nilgiris *viz*, Ebbenadu, Honnathalai, Kodappamand and Kothagiri terrace for a period of 13 months from January, 2004 to January, 2005 at monthly intervals. The present results of the study revealed that the population density of all the four variants of this species in four studied sholas was not changed much over the period of study. However, major change in population size has been noted across the variants in the studied forests. Further it was observed that the recruitment of individuals of all the four variants of *G. fragrantissima* through rhizomatous sprouting was considerably higher than that recruited through seeds.

Keywords : *Gaultheria fragrantissima*, Ecological variants, Population flux, Nilgiris, Western Ghats.

INTRODUCTION

The present study species, *Gaultheria fragrantissima* (family, Ericaceae) is a medicinal shrub distributed from Nepal to Bhutan at the forests of high altitudes from 1800 to 2500m in Central and Eastern Himalayas and in the high hills of Nilgiris at the altitude of 1500m above msl (Anonymous,1956). In Nilgiris it is generally growing at the margins of shola forests (Meher-Homji, 1975). The oil extracted from the leaves popularly known as Indian winter green oil holds many medicinal importances due to the presence of an active principle, methyl salicylate. It is used for the treatment of rheumatism, arthritis, sciatica and neuralgia and in the preparation of pain balms, ointments and perfumes (Chopra, 1932; Polunin and Stainton, 1984). In Nilgiris, four different populations (ecological variants) with different leaf shapes *viz.*, ovate, lanceolate, elliptic-lanceolate and oblanceolate of this species are present in the sholas (Paulsamy *et al*, 2006). In the present investigation, the flux of population density of four ecological variants of the species, *G. fragrantissima* was observed over a period in four major shola forests such as Ebbenadu, Honnathalai, Kodappamand and Kothagiri terrace.

MATERIAL AND METHODS

The study was carried out at monthly intervals for a period of 13 months from January, 2004 to January, 2005. The observations on population flux were made as per the method of Wankhar and Tripathi, (1993). In the respective sholas of occurrence of variants, five permanent quadrats of 1m² were delineated. In case of adults, each plant with a height of one feet was considered as an individual. For the study of seedling population, seedling cohorks were marked in each of the five permanent quadrats in the January month of the years, 2004 and 2005. Individuals arising from the rhizome and seeds were marked with point of different colours. The survival of adults and seedlings were recorded at monthly intervals. The results were expressed in 100m² scale.

RESULTS AND DISCUSSION

A schematic summary of the population flux of four leaf variants of the species, *G. fragrantissima* in four shola forests in Nilgiris such as Ebbenadu, Honnathalai, Kodappamand and Kothagiri terrace in Nilgiris, the Western Ghats is shown in Figs.1-4. The population density of the respective leaf variant was varied widely across the sholas studied. It was further observed that population of lanceolate leaf variant of *G. fragrantissima* was higher (69/100m² in Honnathalai) followed by ovate leaf variant (68/100m² in Ebbenadu), Oblanceolate leaf variant (48/100m² in Ebbenadu) and elliptic- lanceolate leaf variant (47/100m² in Kothagiri terrace). It indicates that generally the microclimatic conditions existing in Ebbenadu shola forest provide suitable niche to the variants particularly to ovate and oblanceolate leaf variants. Despite the changes in population density during subsequent sampling, each variant in all the sholas maintained their early count at the end of the study period. This annual maintenance of population of this type of wild species may be attributted to climax formation of the sholas in Nilgiris (Bor, 1942; Champion and. Seth, 1968; Singh, 1994). Further, it is observed that the recruitment of individuals of all the four variants through rhizomatous

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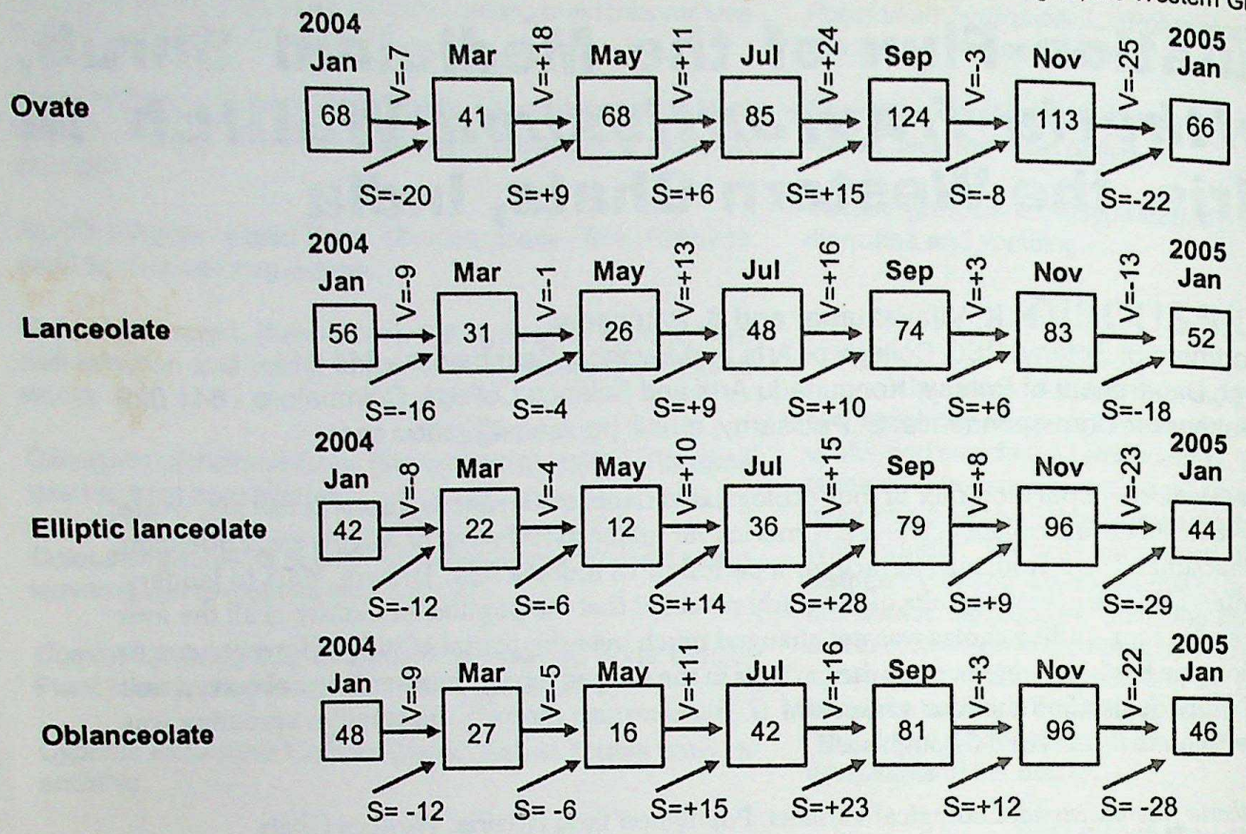

Fig.1. Schematic summary of the population flux of ecological variants of *Gaultheria fragrantissima* in Ebbenadu shola during January, 2004 - January, 2005. Numbers in boxes refer to the population density (plants / 100m²) and numbers written along the arrows refer to the population recruited vegetatively (V) and through seedlings(S) +, gain; –,loss.

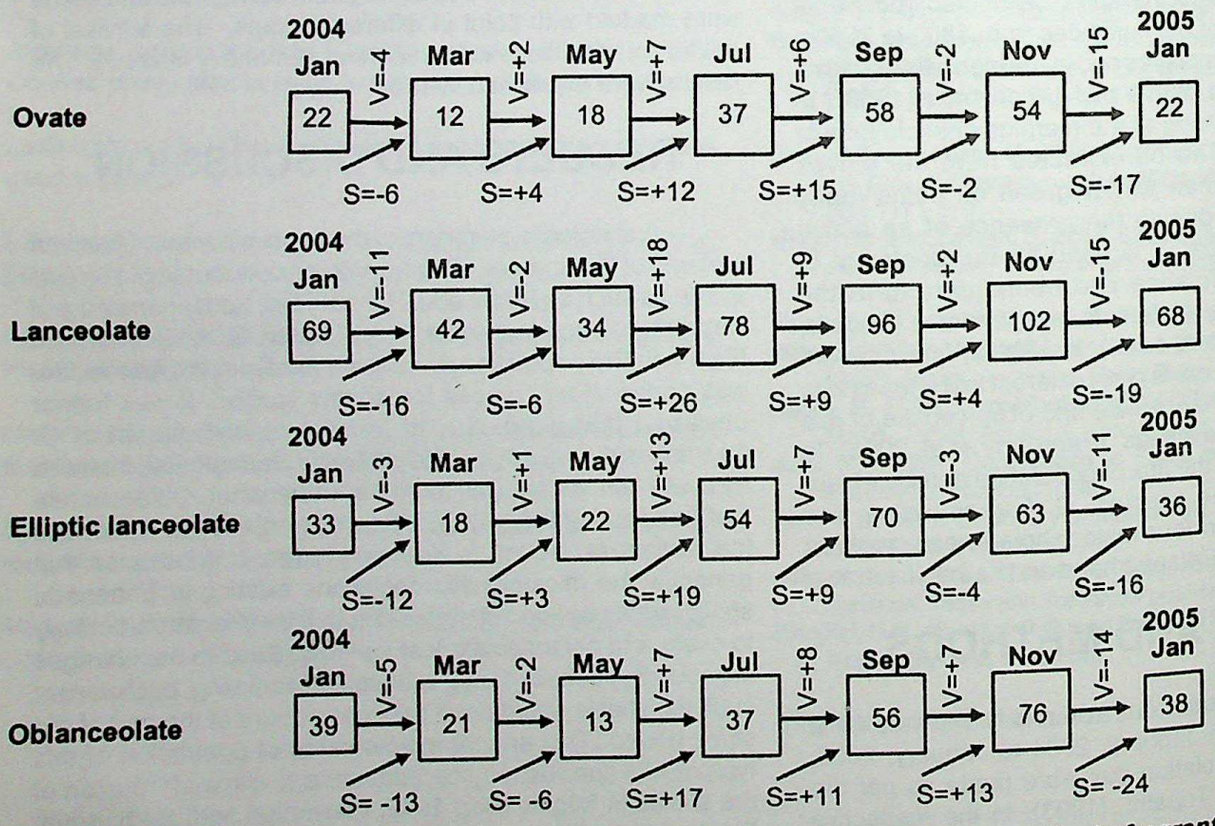

Fig.2. Schematic summary of the population flux of ecological variants of *Gaultheria fragrantissima* in Honnathalai shola during January, 2004 - January, 2005. Numbers in boxes refer to the population density (plants / 100m²) and numbers written along the arrows refer to the population recruited vegetatively (V) and through seedlings(S) +, gain; –,loss.

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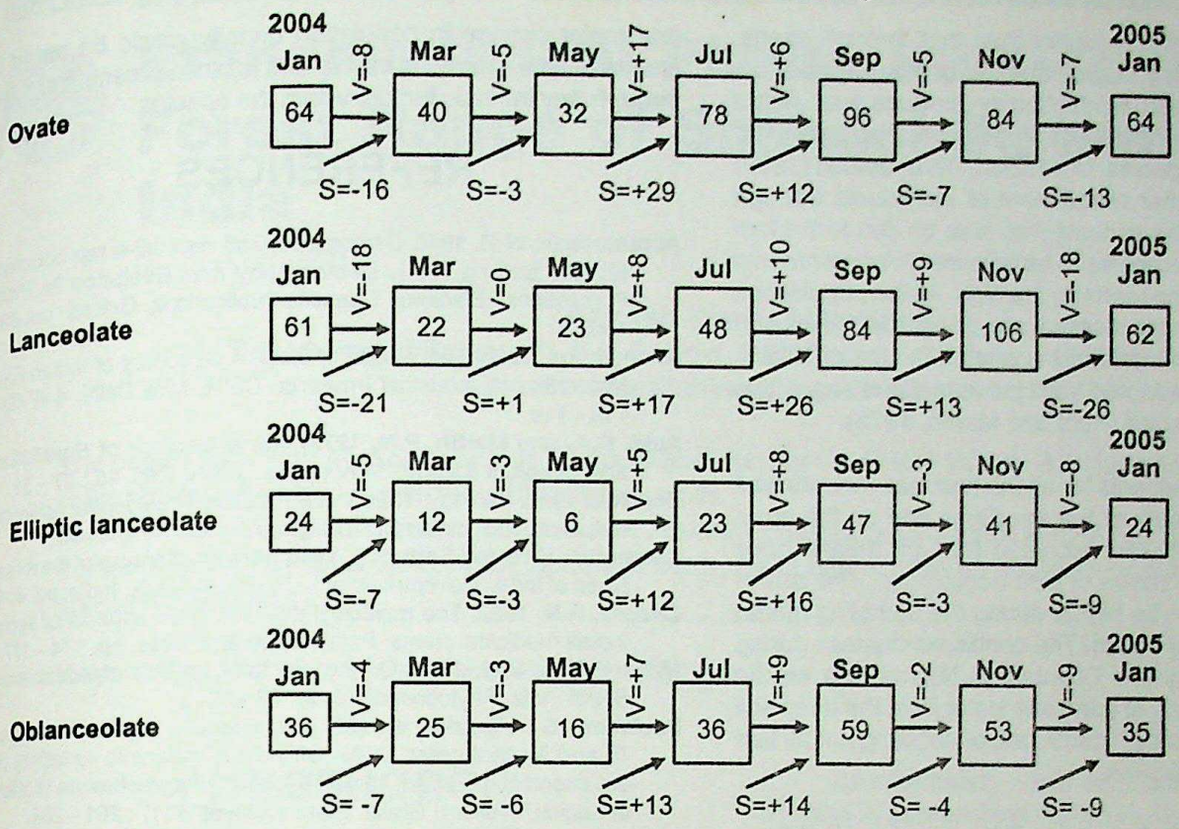

Fig.3. Schematic summary of the population flux of ecological variants of *Gaultheria fragrantissima* in Kodappamand shola during January, 2004 - January, 2005. Numbers in boxes refer to the population density (plants / 100m²) and numbers written along the arrows refer to the population recruited vegetatively (V) and through seedlings(S) +, gain; –,loss.

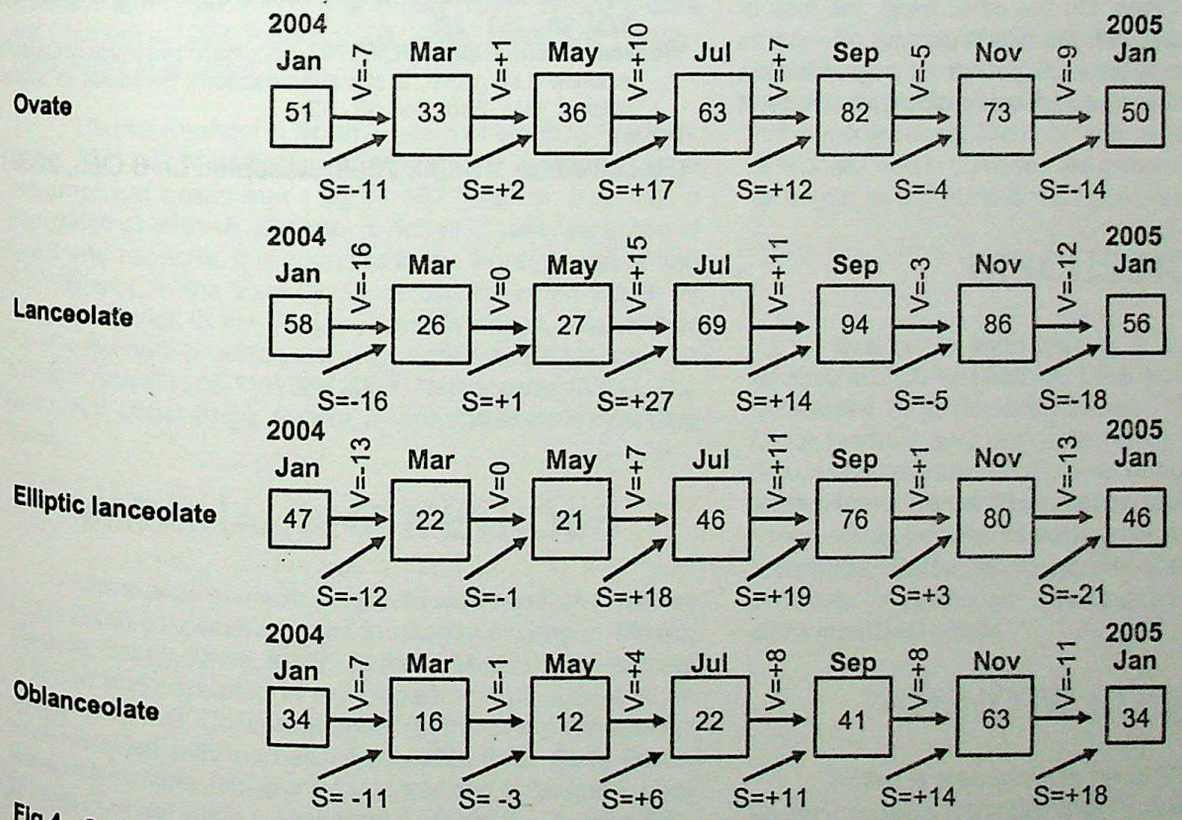

Fig.4. Schematic summary of the population flux of ecological variants of *Gaultheria fragrantissima* in Kothagiri terrace shola during January, 2004 - January, 2005. Numbers in boxes refer to the population density (plants / 100m²) and numbers written along the arrows refer to the population recruited vegetatively (V) and through seedlings(S) +, gain; –,loss.

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sprouting was considerably higher than that through seeds. Sundriyal *et al.* (1987) stated that high altitude species generally have low seed production or produce less viable seeds and it is compensated by vegetative propagation to insure survival of the species. In addition, Abrahamson (1980) explained that the higher recruitment of individuals through vegetative means by the variants may also be due to the fact that clonal multiplication seems to be favoured in environments where seed and seedling mortality are high. All the populations of the species, *G. fragrantissima* are generally growing in partially shaded environment in the shola margins of Nilgiris and this fact of shade-adapted habit induces active vegetative propagation in this species (Auld and Martin, 1975).

The loss of individuals for all the four leaf variants was greater through the death of the seedlings and on the other hand the adults which were recruited through means have established well in the sholas of their occurrence. The loss of seedlings was noted to be higher during the sampling month of January in the study period. The continued dryness during the months of January and February in Nilgiris may not be favourable for the supply of adequate water from the deep soil which may result in the high death rate of seedlings of all leaf variants of *G. fragrantissima*.

From the overall observation, it is understood that there is no external threat to all the four variants of *G. fragrantissima* as they maintained their population without any major change in population density. The recruitment of individuals by all the four leaf variants of this species in four studied sholas of Nilgiris is mainly through rhizomes. On the other hand, the loss of individuals is mainly met with the seedlings only. Hence as per the concept of climax, the wild species, *G. fragrantissima* maintained its population size constantly with fluctuations. If any major change in population density would be noted in course of time, it may be mainly due to biotic pressure and not through any intrinsic factors within the species.

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Ethnomedicinal Plants Studies in Jaitpur Forest Range of Shahdol District, Central India

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The paper deals with 41 Angiospermic plants species having medicinal values. Attempts have been made to collect information by approaching different agencies.
Shahdol is one of the hilly and tribal district of Madhya Pradesh large numbers of plants have been used in different disease by local inhabitants. Botanical name, local name, families and uses in different disease are given parenthetically.

Keywords : Medicinal Plants, Agnivesa, Tribe, Jaitpur forest range.

INTRODUCTION

During exploration of the flora of Madhya Pradesh the auther has found many plants that are used medicinally by local people. A number of workers have published useful ethnobotanical accounts pertaining to different parts of Madhya Pradesh, Jain 1963, Brijlal and Dubey 1992, Tiwari, Mishra and Chatterjee 1996, Pathak 2003, Khan, Singh and Pandey 2005; Khan *et al*, 2008.

District Shahdol is North Eastern of Madhya Pradesh, under 23°17'47'N latitude and 81°21'21"E longitude. Total geographical areas sums up to 5671 sq/km. and has a population of 908148. Shahdol is riched in vast resources of forest and minerals. It is bounded in the North by Satna and Sidhi district, in the East by Korea district, in the South by Anuppur district, in the West by Umaria district. The area is full of water springs which come out on the top hill slopes. The average rainfall is 85.11% and above temperature 13.6°C. The tribes, *Kol, Gond, Baiga, Panika, Bharia* and *Muria* are residing there.

MATERIAL AND METHODS

The survey of medicinal plants was conducted for two (2008-2009) consecutive years in villages of Jaitpur, Bhatia, Mantolia, Bairag, Khora, Kudeli, Mahora. Medicinal plants were collected and deposited in the Department of Botany of Pt. S.N.S. Govt. P.G. College Shahdol (M.P.). The plant species were identified with the help of available flora, State Forest Research Institute Jabalpur (M.P.) India and Botanical Survey of India (Central Circle), Allahabad (U.P.) India, Tiwari 1968.

All information about ethnobotanical plants has been collected by approaching the local sadhus, vaidyas, old people, plant collectors, *Dravya Oshdi Chart* and *Chark Sanhita* of *Agnivesa*.

RESULTS

Forty one families encompassing 41 plants species have been reported which are used medicinally by local peoples. Out of families 30 belong to *Dicotyledons* and 1 family to *Monocotyledons*. The botanical names, local names, families and parts used in diseases are given in Table -1. Families area arranged as per Bentham & Hookers classification.

DISCUSSION

During the investigation, it was found that 41 plant species and 30 families are used as herbal medicine for the treatment of various diseases. There are 29 families Dicotyleadons and only one family Monocotyledons. The discussion revealed local name of species, plant part used, formulation of herbal drug used by traditional healers and tribal communities for species such as, *Argemone mexicana, Aegle marmelos, Butea monosperma, Carria fistula, Calotropis procera, Asparagus racemosus* etc. in there folk ethmonedicinal plants.

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Table 1 : Ethnomedicinal uses of plants in Jaitpur forest range.

(DICOTYLEDONS)

S.N.	Family	Botanical Name	Local Name	Part Used
1.	Apiaceae	*Centella asiatica* (L). Urban	Bramhi	Entire plant used as nervine tonic, and skin diseases as weak sedative, cardio depressant, hypotensive and in leprosy.
2.	Asteraceae	*Eclipta prostrata* L.	Kala Bhegraj	Entire plant tonic given in spleen enlargement and leaf juice given in eczema and as hair tonic.
3.	Apocynaceae	*Rauvolfia serpentina* (L.) Benth.	Sarapgandha	Leaf juice is used as remedy for opacity of cornea. Root is sedative, reduces hypertension.
4.	Asclepiadaceae	*Calotropis procera* Br.	Madar	Flowers are used in cold, cough and Asthma. Powdered Root bark used in dysentery. Fresh leaves in ulcer and as wormicidal.
5.	Acantahceae	*Justicia adhatoda* L	Arusa	Leaf decoction is used in cough bronchitis and rheumatism.
6.	Acantahceae	*Andrographis paniculata* (Burm. f.) Wall	Kariyat	Entire plant used in treatment of bronchitis, dyspepsia and dysentery.
7.	Acantahceae	*Hygrophila auticulata*	Talmakhana (Schum)	Leaves, seeds and roots are used as diuretics and also for jaundice, dropsy, rheumatism and urinogenital disorder.
8.	Amaranthaceae	*Achyranthes aspera* L.	Chirchitta	Used in cough and its decoction is given in renal dropsy and bronchial infection treatment of phyrrhoea fever, enlargement of liver and spleen.
9.	Cruciferae	*Cleome viscosa* L.	Hurhuria	Leaf juice is used for ear troubles.
10.	Bombacaceae	*Bombax ceiba* L	Semur	Root is stimulant, tonic and chiefly given in impotency.
11.	Combretaceae	*Terminalia arjuna* (Roxb.) Wt. & Am.	Arjun	Bark infusion used in heart troubles and leaf juice in earache, hypertension and as diuretic and has tonic effect in cirrhosis of lever.
12.	Cucurbitaceae	*Coccinia grandis* (L). Voigt	Kundru	Juice from leaves and roots is used in diabetes.
13.	Cactaceae	*Opundtia elatior*. Mil.	Nagphani	Baked fruit is given in whooping cough and a syrup of the fruit increases secretion of bile.
14.	Caesalpiniaceae	*Cassia fistula* L.	Amaltash	Root with black pepper and leaves of kathal applied to cattle for giving relief to swollen throat.
15.	Caesalpiniaceae	*Cassia tora* L.	Chakwda	Leaf and seeds used in skin disease for ring worm and itch.
16.	Convolvulaceae	*Cuscuta reflexa* Roxb.	Amarbel	Seeds used in ulcer and liver disorders.
17.	Dipterocarpaceae	*Shorea robusta* Gaertn f.	Sal	Resin is used in skin diseases, diarrhoea and dysentery
18.	Euphorbiaceae	*Euphorbia hirta* L.	Lal dudhi	Entire plant used in bronchial affections, cough, asthma.
19.	Fabaceae	*Butea monosperma* (Lamk) Tamb	Palas	Seeds are internally administerd as an anthelmintic in treatment of round worms. Gum is used in treatment of diarrhoea.

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20.	Fabaceae	*Dalbergia sissoo* Roxb	Shisham	Decoction of leaf useful in gonorrhoea.
21.	Fabaceae	*Mucuna pruriens* (L) DC	Kemmach	Root used in paralysis seeds are used as nervine tonic, menses troubles and as vermifuge, strong infusion of roots mixed with honey is given in cholera.
22.	Lythraceae	*Lawsonia inerdmis* L.	Mehndi	Lea paste or decoction used as gargle in sore throat, burns, bruises and skin inflammation, Seeds useful in urinary troubles.
23.	Lamiaceae	*Leucas cephalotes* (Wild.) (Roth) Spr.	Gumi	Leaf used as digestive antiseptic and earache and fever. Flowers are used in cough and cold.
24.	Lamiaceae	*Ocimum americanum* L.	Kali tulsi	Leaf decoction is given in Maleria and abdominal diseases.
25.	Menispermaceae	*Tinospora cordifolia* (L.) Merr.	Gurch	Root extract is used in fever, cold cough, as blood purifier, in acidity and jaundice.
26.	Meliaceae	*Azadirachta indica* A. Juss	Neem	Bark, leaf and fruit decoction is antiseptic and used in ulcer, eczema and skin diseases.
27.	Mimosaceae	*Acacia nilotica* (L.) Del	Babul	Bark used in diarrohea, dysentery and diabetes.
28.	Mimosaceae	*Albizdia lebbeck* (L.) Willd	Siris	Bark and seeds given in piles and diarrhoea.
29.	Nyctaginaceae	*Boerhaavia diffusa* L.	Punarnava	Root is laxative and used in asthma, jaundice and dropsy.
30.	Papaveraceae	*Argemone mexicana* L	Pilikateli	Stem latex used in dropsy, jaundice and conjunctivities.
31.	Papaveraceae	*Papaver somniferum* L	Afeem	Flower, fruits and seeds have pain releasing and sleeping effects and useful in irritating cough, pneumonia, ulcers, gastritis and influenza.
32.	Rutaceae	*Aegle marmelos* (L) Corr	Bel	Fruits is given in diarrhoea dysentery irriation of alimentary canal, fever and as tonic and cooling agent.
33.	Sapotaceae	*Madhuca longifolia*(Koen) Mac Br.	Mahua	Decoction of bark is used in incurring bleeding gums and ulcers. Flowers are used in cough and bronchitis.
34.	Solanaceae	*Datura stramonium* L.	Dhatura	Drug consists of dried leaves, flowering tops and seeds are used in treatment of asthma. Stramonium as one of the main ingredients is used as expectorant, antispasmodic, demulcent and anodyne in cough and asthma. (Leaves and seeds are narcotic and sometimes used for criminal poisoning).
35.	Tamariscaceae	*Tamarix diodica* Roxb.	Khirsati	Bark used to cure diarrohea and dysentery.
36.	Verbenaceae	*Clerodendrum indicum* (L) Ktze	Tiraia	Leaf and root used in rheumatism, swellings cough, wounds as antiseptic.
37.	Verbenaceae	*Vitex negundo* L.	Negur	Leaf useful in wounds inflammatory pain and bone fractures, rheumatic swellings of joints.
38.	Zygophyllaceae	*Tribulus terrestris* L.	Gokhuru	Fruits is cooling agent diuretic and used in micrutition, impotency and urinary disorders. Paste prepared from leaves is used for treatment of stones in bladder.

MONOCOTYLEDONS

1.	Liliaceae	*Aloe barbadensis* Mill	Ghikanvar	Leaf juice used as purgative and cosmetic.

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2.	Liliaceae	*Asparagus adscendens*, Roxb.	Safedmusli	Root is used as Demulcent and also in diarrhoea and dysentery.
3.	Liliaceae	*Asparagus racemosus* Willd.	Satawar	Root is used as demulcent, aphrodisiac, diuretic, antidysentric and as demulcent in veterinary medicine.

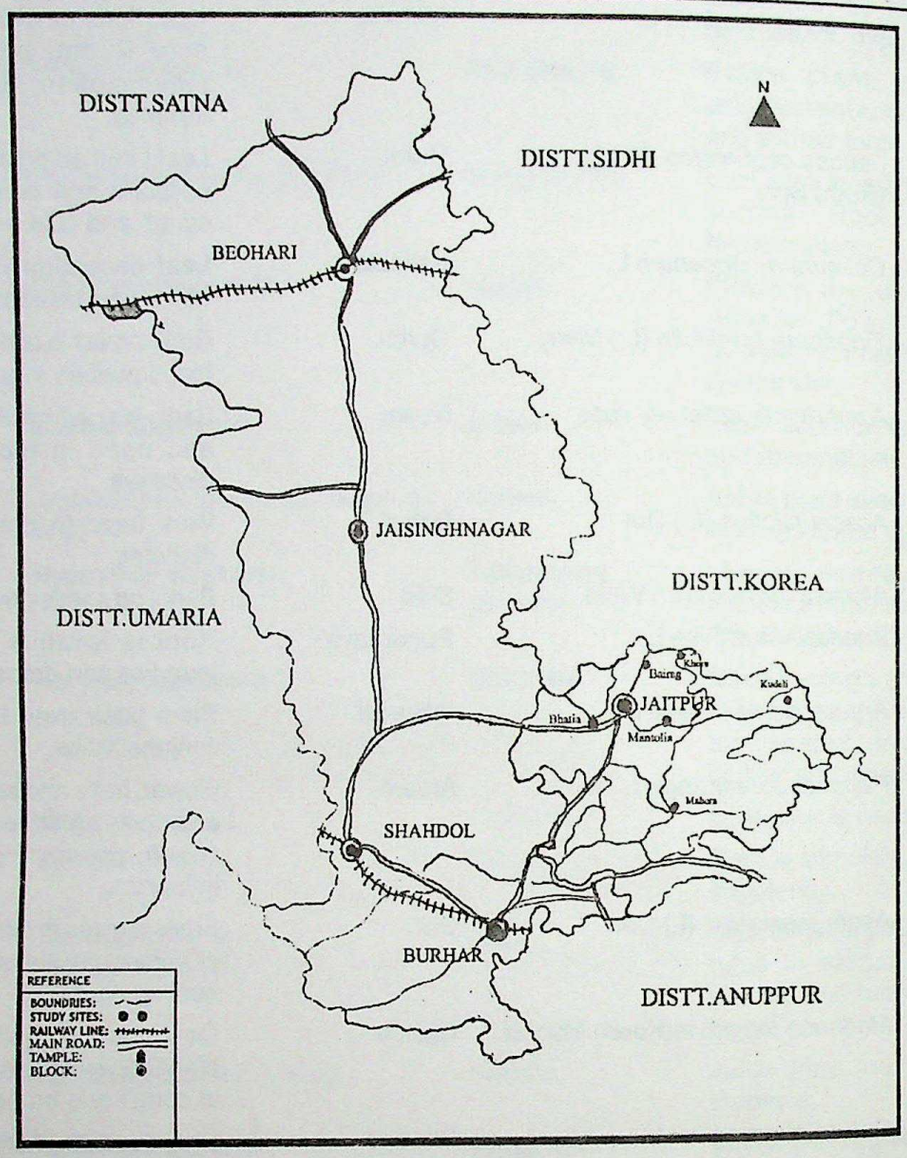

Fig 2 : Location Map of The Study Area Distt. Shahdol (M.P.)

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Ethnomedicinal Importance of Some Trees of Barnawapara Sanctuary of Chhattisgarh, India

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The flora of Barnawapara wild life sanctuary of Chhattisgarh state consists of tropical deciduous forests and several other varieties of trees like Mahua, Tendu, Ber etc. The tribals and local communities dwelling in and around the sanctuary have long used the trees for valuable medicinal substances. An attempt has been made to compile the ethnomedicinal utilization of 18 species of trees belonging to 16 genera of 13 families. Some documented tree species are *Terminalia chebula, T. bellerica, T. arjuna, Ficus hispida, Gardenia gummifera* and *Adina cordifolia* etc. In general fruits, barks, leaves and other parts of the trees are used as medicine for traditional health care. In the recent past, traditional healers are dwindling in big numbers and thereby posing a threat to traditional medicinal practices. To address this issue, it is necessary that proper investigation of medicinal properties of various plants of Barnawapara wild life sanctuary be conducted scientifically.

Keywords : Ethnomedicine, Barnawapara wild life sanctuary.

INTRODUCTION

Barnawapara wild life sanctuary is situated in the northern part of Mahasamund district of Chhattisgarh state. It is located 85 km away from Raipur city, the capital of Chhattisgarh. The Barnawapara wild life sanctuary is known for its lush green vegetation along with varieties of medicinal plants and unipue wild life. The primitive tribes residing in the near by villages of Barnawapara forest have established a proven traditional knowledge system of self-healing based on bio-health. The communities utilize a large number of plant species for herbal medicine which is naturally made available in the Barnawapara sanctuary (Tiwari, Jadhav and Kunjam, 2008).

Herbs and herbal remedies are known to Indians from the Vedic period. From time immemorial mankind is dependent on plants as a source of food, medicine, fibre and fodder; ect. and consider them to be magico-religious with socio-cultural significance. This knowledge intimates relationship between man and plants in his immediate surroundings and is being passed on to us mainly through surviving traditions (Jain, 2004).

Area of Study : The Barnawapara wild life sanctuary is situated 85 km away from the capital city of Raipur, which falls under the Raipur forest range of Chhattisgarh state and is relatively a small one covering an area of only 246 sq. km. The topography of this region comprises of flat and hilly terrain with altitude ranging between 265 to 400 mts. The climate here is tropical, with summer being usually hot. Vegetation is dry deciduous forest with Teak, Bamboo, Sal, Terminalia and other miscellaneous species. This sanctuary also has a large number of wildlife such as Chitah, Gaur, Leopard, Neelgai etc. However, the main attractions are the Sloth bears and the Tigers. This is the reason why Barnawapara wild life Sanctuary is slowly but firmly emerging as a spot of ecotourism.

MATERIAL AND METHODS

The method used in the recent study conducted during the period of October 2009 to March 2010 was to collect different parts of ethnomedicinal plants and related pictures from the Barnawapara sanctuary which was the primary input for the project. The herbal specimens prepared are deposited in the Deptt. of Botany, Govt. V.Y.T.P.G. Auto. College, Durg (C.G.). The data is recorded on the scientific names, local names, part (s) used, method of drug preparation, and dosages of plants parts used for different ailments. These were derived based on observations, queries and interviews with local traditional healers and villagers of different localities of the sanctuary. The specimens were identified with the help of literature - (Haines 1921-25, Hooker 1872-1897, Duthie 1961, Kirtikar and Basu, 1918 and Verma, *et al.* 1985).

Enumeration : The species are arranged and described in alphabetical order with the botanical name, family, local name, plant part (s) used, method of drug preparation, dosage and dose administration.

1. ***Azadirachta indica* A. Juss. (Meliaceae) 'Neem'**
   Leaves - Decoction used to expel the worms from intestine. Leaves are boiled in water and used as a disinfectant. Bunch of leaves are placed on the bed of the patient of small pox for cooling effect.
   Seed - Oil is locally applied to cure skin diseases.
   Twigs - Used for cleaning teeth.
2. ***Acacia nilotica* (L.) Del. (Mimosaceae) 'Babul'**
   Leaves - Paste of young tender leaves cures malignant

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tumour.
Twig - Used to cure teeth trouble.
Root - Paste is applied on skin diseases.

3. ***Adina cordifolia* (Salisb.) Hook. (Rubiaceae) 'Karam'**
Bud - Small buds are ground with black pepper and sniffed in to the nose in case of severe headache.
Bark - Paste is applied locally on scars of skin. Bark juice is used to kill worms in sores.

4. ***Anacardium occidentale* Linn. (Anacardiaceae) 'Kaju'**
Bark - Used to cure malaria, when quinine fails.
Fruit - Juice is administrated to cure ulcers, to remove warts and corns and also to treat various skin infections.

5. ***Bauhinia variegata* L. Fabaceae (Caesalpinideae) 'Kachnar'**
Fruit - Used as a medicine for indigestion and cough.
Bark - Used as a anthelmintic and is to cure various skin diseases.

6. ***Dalbergia sissoo* Roxb. Fabaceae (Papilionaceae) 'Sissoo'**
Root - Decoction is applied locally to cure gonorrhoea.
Leaf - Crushed young leaves mixed with water and sugar cubes, taken orally to cure excessive bleeding during menstruation.

7. ***Diospyros melanoxylon* Roxb. (Ebenaceae) 'Kendu'**
Root and stem - Decoction of bark administered orally for post natal pains.
Fruit - Powderd fruit is taken orally in stomach disorder. It is externally applied on piles.

8. ***Ficus hispida* L.F. (Moraceae) 'Doomar'**
Latex - Applied locally on cuts and for headache. It is orally taken to cure urinary infection.
Receptacle - Past is locally applied on face to cure swelling due to heat.

9. ***Gardenia gummifera* L. (Rubiaceae) 'Kurdu'**
Leaf & Bark - Decoction is taken orally to cure sexual diseases.

10. ***Lagerstroemia flos-reginae* Retz. (Lythraceae) 'Jharul'**
Fruit - Extract used to cure diabetes.
Bark - Juice taken orally in stomach pain and diarrhoea.

11. ***Melia azedarach* Linn. (Meliaceae) 'Bakain'**
Leaf - Juice is used to expel worms from intestine.
Bark - Oil is applied in rheumatism.

12. ***Polyalthia longifolia* Benth. (Anonaceae) 'Bakain'**
Bark - Stem bark extract lowers blood pressure. Powder of stem bark mixed with butter which is applied locally to cure gonorrhea.

13. ***Sterculia urens* Roxb. (Sterculiaceae) 'Kullu'**
Leaf - Freshly prepared paste of leaves applied locally on chapped and cracked skin in winter.
Seed - Oil is used to remove worms from animals intestine.
Gum - Mixed with milk and given orally to cure piles.

14. ***Syzygium cumini* Linn. (Myrtaceae) 'Jamun'**
Bark - Crushed stem bark is taken orally with cold water twice a day for a week for successful treatment of dysentery. It is also used as antidote to snake bite and scorpion sting.
Seed - Powdered seed is taken orally to cure diabetes.

15. ***Terminalia belerica* Roxb. (Combretaceae) 'Bahera'**
Fruit - Unriped fruits are used as laxative. Dried fruit powder is taken orally in fever. It is a part of Triphala.

16. ***Terminalia chebula* Retz. (Combretaceae) 'Harra'**
Fruit - Unriped dried fruit is chewed in stomach disorder.

17. ***Terminalia arjuna* W. & A. (Combretaceae) 'Kahua'**
Stem - Dried & powdered stem bark is used twice a day for two weeks as cardiac tonic.

18. ***Vitex negundo* Linn. (Verbenaceae) 'Nirgundi'**
Leaf - Leaf oil is applied on inflammatory swelling of joints in rheumatism. Leaves and twigs are boiled in water and are used to take warn bath by women after seven days of delivery.
Seed - A laddoo of fried seeds eaten daily to treat dropsy.

RESULTS AND DISCUSSON

18 tree species belonging to 16 genera and 13 families have been recorded in the present work. Tree species presented in this study needs special attention on account of their restricted availability, threatened status and ethnomedicinal significance. The present investigation has highlighted the therapeutic value of trees as a cure for different diseases. The trees of Barnawapara are extremely important natural resource for the country, which plays a crucial role both in economic and social life of the local people. The medicinal plants listed above should be subjected to intensive phytochemical screening and pharmacognosy in search of new leads for the modern herbal drugs.

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Ethnomedicinal Plants used Against Diarrhoea and Dysentery in Deograh District of Orissa (India)

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This paper attempts to document the traditional knowledge on 21 ethnomedicinal plants belonging to 17 families. The Binomials with thir local names, family, methods of preparation, usages and mode of administration of drugs from the plants are discussed.

Keywords : Ethnomedicinal plant, Diarrhoea, Dysentery, Deogarh District.

INTRODUCTION

Medicinal plants are commonly used by the ethnic communities throughout the would for treating their day to day ailments and uses of many have been brought into the modern system of medicine. From Indian Sub-continent, enough ethnomedicinal data have been collected, which may be useful for chemical, pharmacological and clinical analysis and testing (Shah, 2008). Tribals are distinct ethnic groups living in forests, hills and are termed as *'Adivasis'*. They use plants found around them to cure various ailments or diseases. Their knowledge of plants has descended from one generation to another as a domestic practice (Brahmam, 2000). Herbal drugs are used widely to avoid the occurrence of toxic side effects due to long term usage of synthetic drugs during treatment of chronic diseases (Sandhyarani and Jagmohan, 2009).

Deogarh district is one of the 30 districts of Orissa situated in western part of the State. It is at an altitude of 250m above sea level having a forest area of about 68996.41 Ha. The climate is generally tropical with one rainy season (from June to September) with an average rainfall of 1445.3 mm. The temperature in hot season is approximate 46°C and in cold season 8°C. Due to its unique topography and variable climatic conditions, the district is enriched with diversified medicinal flora. The District is situated between 21° 32'-21° 35' North Latitudes and 84° 41'-84° 45' East Longitudes. It is bounded on the North by Sundargarh District, on the East and South by Angul District, on the West by Sambalpur District of Orissa (Fig.1). From geographical point of view, the district is full of hilly forestes, elevated plateaus and plains embracing rich forest pockets.

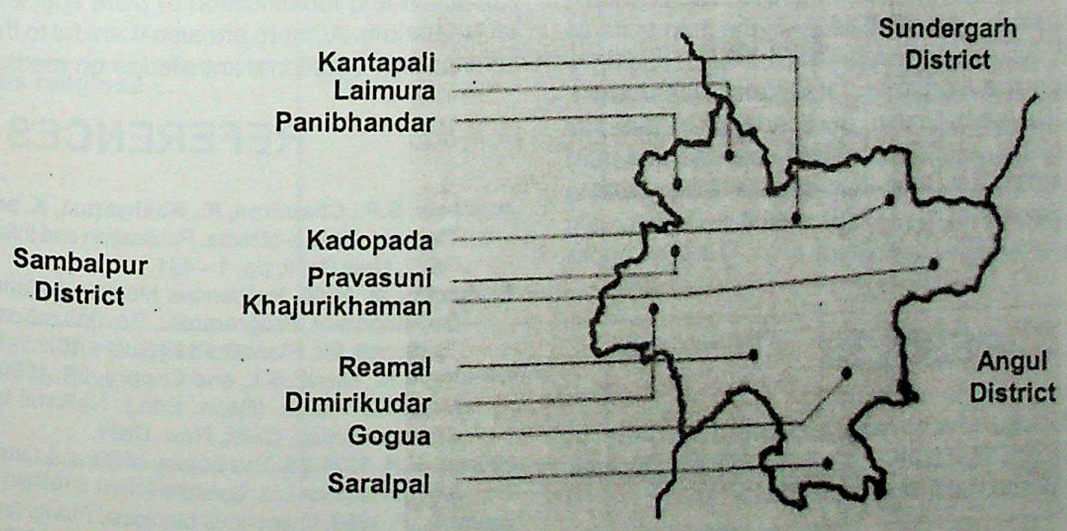

Fig. 1 : District Map of Deograh Showing Surveyed Areas (Spotted)

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Plate A : A Bhuiyan tribe woman in front of her cottage

Plate B : A Kolha tribe man in front of his cottage

Deogarh district is inhabited by a number of tribal people numbering approximately 74,209 which is about 27.07% of the total population of the district. The tribal communities include *Kishan, Kolha, Khaira, Gond, Bhuinya, Sabara, Munda, Kharia, Oraon, Paudi Bhuinya* and *Mankadia. Kishan, Gond,* and *Bhuinya* are dominant tribes of the district (Plates A & B). The tribal as well as non-tribal group of people residing in villages use medicinal plants available around them for treatment of various diseases. The present study highlights those plants used by them to cure diarrhoea and dysentery.

MATERIAL AND METHODS

The authors have conducted extensive field survey during 2007-2009 in the tribal belts and other interior villages, adjoining forest areas in the district to collect information about plant species of Ethnomedicinal value. Local herbal medicine Practioners and different tribal communities were interviewed to get information about medicinal uses of these plants. Collected plant species were identified with the help of floras (Haines, 1921-25; Saxena and Brahmam, 1994-96) and have been preserved in the Herbarium of Botany Department, Deogarh college, Deogarh, Orissa. To ascertain the medicinal values of these plants, earlier published scientific literature sources were referred (Rai Choudhury *et al.,* (1975); Saxena & Dutta (1975); Saxena *et al.,* (1979); Kaushik (1988); Jain (1991); Kirtikar & Basu (1991); Ambasta *et al.,* (1992); Chopra *et al.,* 1996).

RESULTS

The medicinal plants are tabulated serially in alphabetical order with their botanical names followed by their local names, family and medicinal uses (Table-1).

DISCUSSION

The present study on uses of medicinal plants for treatment of diarrhoea and dysentery in Deogarh district (Orissa) is based on informations collected from the traditional healers and other herbal medicine practioners. During the study 21 plants belonging to 21 Genera and 17 Families were identified which are discussed in detail with mode of their administration. The survey of remote areas reveals that the people of the area possess tremendous knowledge of herbal drugs and their healing properties for treatment of different ailments. For the benefit of society, the reported plant species should be taken care and also steps be taken for their conservation as well as cultivation. Further investigations at biochemical level are needed to ascertain the active principles present in the plants before recommending them for use by the elite.

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Table 1 : List of plant species used by the tribals and rural folk of Deogarh district and their medicinal uses for treatment of diarrhoea and dysentery.

S.No.	Botanical name	Local Name	Family	Medicinal uses
1.	**Aegle marmelos** (L) Corr	Bel	Rutaceae	Pulp of ripe fruit with water and sugar are taken once a day for 7- 8 days to cure dysentery. 5 g of dried pulp of raw fruits are taken orally twice a day for 3-5 days to cure diarrhoea.
2.	**Alangium salvifolium** (L.F.) Wang.	Ankul	Alangiaceae	For diarrhorea, 3 grams of root bark are pounded and are taken with rice washed water and honey once after each loose motion. For dysentery 6 g of leaf are crushed to make a paste and taken orally with water once a day for 3-5 days.
3.	**Aloe barbadensis** Mill.	Ghikuanri	Liliaceae	Leaf of the plant is kept in cold water for some time. Then juice of leaves is taken two times a day for 2-3 days to cure dysentery.
4.	**Aphanamixis polystachya** (Wall. Parker)	Gilakusum, Pani kusum	Meliaceae	2-3 leaflets are crushed and taken twice a day for 3 days as a treatment for blood dysentery.
5.	**Azadirachta indica** A. Juss.	Neem	Meliaceae	One spoon of seed powder twice a day for 3 days is prescribed to cure diarrhoea. For treatment of dysentery, the stem bark is mixed with water. Half a cup of it taken at an interval of 3 hours for 2-3 days.
6.	**Bauhinia variegata** L.	Kanchana	Caesalpiniaceae	Stem bark is crushed in to paste and taken roally once a day for 3 days for chronic dysentery.
7.	**Bombax ceiba** L.	Simili	Bombacaceae	Stem bark is crushed and mixed with unboiled milk and is taken orally 2 times a day for 3 days for treatment of diarrhoea.
8.	**Butea monosperma** (Lam.) Taub.	Palasa	Fabaceae	Resin of the plant mixed with water is taken orally 2 times a day for 3-5 days to check diarrhoea.
9.	**Euphorbia hirta** L.	Chitakuti	Euphorbiaceae	5-7 leaves powdered with one gram fruit of *Myristica fragrance* are taken 2 times a day for 3-5 days to cure infantile dysentery.
10.	**Ficus religiosa** L.	Peepal	Moraceae	One spoon powder of stem bark mixed with two spoons of juice of Jamun (*Syzygium cuminii*) leaf is taken 2 times a day for 3 days for diarrhoea and dysentery. 25 g of tender prop roots are taken and crushed. It is mixed with rice washed water and curd & is taken orally once a day for 2-3 days to cure dysentery.
11.	**Holarrhena pubescens** Wall. ex Don.	Kurei	Apocynaceae	Stem bark is crushed with water and is taken once a day for 2-3 days to cure diarrhoea. ½ cup of decoction of stem bark with honey is taken 3 times a for 3-5 days to cure dysentery.
12.	**Mangifera indica** L.	Am, Amba	Anacardiaceae	Stem bark is crushed into paste and taken orally once a day for 5-7 days for dysentery.
13.	**Mimusops elengi** L.	Baula	Sapotaceae	5 matured fruits are pounded to make a paste and it is mixed with one spoon of sugar and is taken 2 times a day for 5 days to cure dysentery.

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14.	**Mucuna pruriens** (L.) DC.	Baidanka	Fabaceae	Half tea spoonful of the seed powder is taken once a day for 3 days for treatment of diarrhorea.
15.	**Paederia scandens** (Lour) Merr.	Pasaruni	Rubiaceae	250 g of leaf and 200 g of unboiled rice are crushed, boiled and taken orally with gur (molasses). The dose is continued once a day for ten days to cure diarrhoea and dysentery.
16.	**Papaver somniferum** L.	Aphima	Papaveracea	Latex of the fruit is taken once a day for 2-3 days for chronic dysentery.
17.	**Phyllanthus emblica** L.	Amla	Euporbiaceae	Stem bark is crushed into a paste and is taken orally once a day for 3-4 days for diarrhoea and dysentery. 4-5 spoons of leaf juice mixed with one glass of milk is taken 2-4 times a day for 2-3 days to cure diarrhoea. Decoction of the stem bark mixed with 2-3 g of seed powder of *Trigonella foenum-graceum* is taken twice a day for 5 days to cure dysentery.
18.	**Sida cordifolia** L.	Bisiripi	Malvaceae	Decoction of plant mixed with one spoon of powdered fruit of Nutmeg (*Myristica fragrans*) is taken 2 times a day for 3 days to cure diarrhoea.
19.	**Syzygium cuminii** (L.) Skeels	Jamu	Myrtaceae	Juice of the stem bark mixed with goat milk is prescribed orally once a day for 3 days to cure diarrhoea. Two spoons of stem bark juice mixed with ½ spoon of honey is taken once a day for 3 days as antidysenteric. Equal amount of tender leaves of *Syzygium cumini, Mangifera indica* and *Punica granatum* are taken and juice is extracted and it is taken with honey once a day for 3-5 days to cure infantile diarrhoea.
20.	**Terminalia bellirica** Roxb.	Bahada	Combretaceae	Latex of the plant mixed with honey is administered 3 times a day for 2-3 days to cure diarrhoea.
21.	**Tamarindus indica** L.	Tetel, Kanyan, Tentuli	Caesalpiniaceae	3 g of seed powder mixed with curd are taken 2 times a day in the morning and evening for 3 days to cure diarrhoea

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Ethnomedicinal Plants used to Cure Spermatorrea in Kangra District (Himachal Pradesh)

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The present paper highlights 17 medicinal plants used to cure spermatorrea (locally called Dhat) among tribal people of Kangra valley. In ethnobotanical survery, we collected 17 medicinal plants belonging to 12 Angiospermic families. A brief important ethnomedicinal uses of medicinal herbs, local name and mode of treatment have been reported.

Keywords : Himachal Pradesh, Medicinal plants, Spermatorrea, Tribal people.

INTRODUCTION

Traditional knowledge of human health and medicines has recently become a major global concern. Traditional knowledge of medicinal plants is now considered to play a vital role in addressing the health care needs of developing countries and indigenous people. In India, about 2,500 plant species belonging to more than 1,000 genera are used by traditional healers and about 500 plant species are used by 159 different Pharmaceutical Companies (Chandel *et al.*, 1996). According to W.H.O. Report (1978) on traditional medicines, indigenous treatment is the main source of primary health care system of 80% population of the world.

Studies on medicinal plants in Himachal Pradesh and other places have been carried out by Gupta (1964), Shabnam (1964), Chauhan (1999), Gupta and Gupta (2006) in J & K, Dhiman (2003) in Uttranchal, Tomar and Singh (2005) in U.P. and Verma *et al.*, (2008) in Eastern U.P. However, studies on medicinal plants in Kangra valley (H.P.) are scanty except for a few reports (Ahluwalia, 1952 and Chauhan, 1988).

Therefore, in the present paper an attempt has been made to enlist the medicinal plants used for the treatment of spermatorrea problems in Kangra valley of Himachal Pradesh.

MATERIAL AND METHODS

The present study was conducted as per Standard Methods (Srivastava *et al.*, 2008 a and b.). An extensive ethnobotanical field tour was conducted in rural and tribal localites *viz.* Baijnath, Beer, Utrala, Bandla, Chamunda, Thriyund, Jawalamukhi, Shahpur, Thural and adjacent area of Kangra district during March 2008 to March 2009. We collected 17 plants used to cure spermatorrea. The tribals/rural and elderly people accompanied us and helped during survey, collection and documentation of plant. The information about the local name, ethnomedicinal use of plants for various diseases and the parts as well as mode of treatments was recorded. The collected plants were identified correctly with the help of available literature *i.e.*, Chowdhery and Wadhwa (1984), Badola and Pal (2002) and Atul *et al.*, (2002) and by comparing the specimens with the Herbarium of "The Institute of Research in Indian System of Medicine, Joginder Nagar, District Mandi (H.P.). The herbarium specimens were maintained in the department of botany for further use.

RESULTS AND DISCUSSION

District Kangra, which is in North Western region of Himachal Pradesh is having a large population of tribal and non-tribal people including Gaddi, Gujjar, Dhogri, Bungali, Bhangolia, Labana, Sippi, etc. They have vast knowledge of herbal treatment for a wide range of physical ailments. The herbal treatment practices are comparatively cheap, time saving and provide immediate relief upto some extent. This work is specially focused on documentation of ethnomedicinal uses of plants for curing spermatorrea. A brief account of ethnomedicinal plant species (5 trees, 5 herbs, 4 shrubs and 3 climbers distributed over 12 Angiospermic farmilies) traditionally used for the treatment of spermatorrea is given in Table-1.

CONCLUSION

The district Kangra has been recognised as a rich store house for herbal medicines. Through ages, people of the area has been utilizing this plant resource to cure various ailments. Present study suggestes increasing demand of medicinal plants has resulted in rapid dwindling of these natural resources and there is a urgent need of conservation and sustainable development of medicinal plants. There are some plants which

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Table 1 : Ethnomedicinal herbs used for spermatorra problems.

S.No.	Botanical Name	Local Name	Family	Ethnomedicinal Uses
1.	*Aegle marmelos* (L) Corr.	Bel	Rutaceae	Powder of ripe fruit feed orally with water in early morning helps to cure spermatorrea. Leaf extract also used for this purpose.
2.	*Aloe barbadensis* Mill.	Kuare, Kumari	Liliaceae	Fresh juice of leaves used orally to cure spermatorrea.
3.	*Asparagus adscendens* Roxb.	Shatawari, Sanspan	Liliaceae	Dry roots powder is used orally with milk to cure spermatorrea.
4.	*Cissampelos pareira* L.	Patindu, Batindu	Menispermaceae	Sap of crushed leaves is used orally to cure spermatorrea.
5.	*Cirsium wallichii* DC.	Brusa	Asteraceae	Fresh roots are cleaned, broken into small pieces and kept in water over night. Next day these are ground in the same water. About 10-15 ml solution is given orally in empty stomach in the morning to cure spermatorrea.
6.	*Phyllanthus emblica* L.	Amla	Euphorbiaceae	Dried fruit powder is used orally in early morning to cure spermatorrea
7.	*Justicia adhatoda* L.	Basunti, Vasa	Acanthaceae	Powder of dry flower is consumed with "Kujja Mishri" and water in early morning to cure spermatorra.
8.	*Polygonatum verticillatum* (L.) All.	Salam Mishri	Liliaceae	Fresh roots are broken into small pieces, kept in water overnight, next day these are ground in the same water. About 10-15 ml solution is given orally in empty stomach in the morning to cure spermatorrea.
9.	*Mucuna pruriens* (L.) DC.	Gajar Bel, Kaunch	Fabaceae	Powder of roasted seed is given orally with water early morning to cure spermatorrea and seminal weakness.
10.	*Sida cordifolia* L.	Drir	Malvaceae	Powder of roasted seeds is mixed in desi ghee and given orally alongwith milk early morning to cure spermatorrea.
11.	*Terminalia bellirica* Roxb.	Bahera	Combretaceae	Dry fruit rind powder is used orally to cure spermatorrea.
12.	*Vernonia albicans* DC.	Sahdevi	Asteraceae	Fresh plant is crushed and sap is consumed with "Kujja Mishri" to cure spermatorrea.
13.	*Oxalis corniculata* L.	Maroli, Changari	Oxalidaceae	Whole plant is crushed and sap is consumed to cure spermatorrea.
14.	*Ficus bengalensis* L.	Bari, Bargadh	Moraceae	Milk of the tree is filled in the sweet "Batasha" and feed in the early morning to cure spermatorrea.
15.	*Ficus religious* L.	Peepal, Bar	Moraceae	Powder of dry fruit is given orally with water in early morning to cure spermatorrea.
16.	*Indigofera linifolia* (L.f.) Retz.	Darani-Jathani	Fabaceae	Leaves are mixed in desi ghee of buffaloes and consumed early in the morning with milk to cure spermatorrea.
17.	*Tinospora cordifolia* (L.) Merr.	Giloe	Menispermaceae	Fresh stem is crushed in the night and kept in the same water over night, in the early morning the sap is consumed alongwith "Kujja Mishri" to cure spermatorrea.

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are panacea for most human ailments. Their documentation and protection of this indigenous knowledge, related to the use of medicinal plant is the need of the hour. Creating awareness among the tribal people of Kangra valley on medicinal properties of these plants, which were very often encounter in their daily life through dissemination of research data with extensive activities will go a long way in conserving nature's priceless gift.

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Epidermal Study of Some Medicinal Plants

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The present investigation relates to the study of epidermal structures of *Aloe barbadensis, Boerhavia diffusa, Catharanthus roseus, Cleome gynandra, Coccina grandis, Coleus amboinicus, Crotalaria laburnifolia, Kalanchoe tubiflora, Leucas aspera, Mirabilis jalapa, Piper betle, Solanum trilobatum, Tridax procumbens and Withania somnifera.* Shape, size and wall features of epidermal cells, types, size and distribution of stomata and epidermal hairs on abaxial surface of the leaves were studied. As these characters have taxonomic significance, they can be successfully utilized in the proper identification of the powdered drugs of these taxa.

Keywords : Medicinal plants - leaves - Abaxial surface - Anatomical features.

INTRODUCTION

The study of epidermal features find their application in the applied sciences such as pharmacognosy, forensic medicine, palaeobotany and ethnobotany. Solereder (1908) published an excellent treatise on Systematic Anatomy of Dicotyledons which described the details of epidermis and its appendages. Ensuing decades saw several publications on the epidermal structures. These include the contributions of Rao (1939), Pant (1940) and Foster (1949). Metcalfe and Chalk published two volumes on the 'Anatomy of Dicotyledons' in the year 1950 which included all the previous works on anatomy including the leaf epidermis. There has been good amount of contribution by anatomists in the last few decades to the field of taxonomy and phylogeny. Studies on the structure of epidermis include several families such as Caryophyllaceae (Paliwal, 1966), Cruciferae (Paliwal, 1967), Asclepiadaceae (Krishnamurthy and Sundaram, 1967), Rubiaceae (Bir Bahadur *et al.*, 1971), Scrophulariaceae (Krishnan, 1985), Convolvulaceae (Kannabiran and Nadanakujidam, 1993) and Lauraceae (Baruah and Nath, 1999), as well as a few genera such as *Begonia* (Dehnel, 1961), *Solanum* (Ahmad, 1964), *Physalis* (Kannabiran and Sethi, 1974), *Convolvulas* (Shah, 1976), *Ipomoea* (Kumaravelu, 1987), *Bacopa* (Kannabiran and Ramassamy (1986) and *Aerva* (Padmini and Shanmukha Rao, 2002). As these investigations have helped in the proper identification of medicinal plants, an anatomical study on the abaxial epidermis of following plants was undertaken:

Aloe barbadensis Mill. (LILIACEAE), *Boerhavia diffusa* L. Syn. *B. repens* (NYCTAGINACEAE), *Catharanthus roseus* (L.) Don. Syn. *Lochnera rosea* (L.) Reichb. (APOCYNACEAE), *Cleome gynandra* L. Syn. *Gynandropsis pentaphylla* DC (CAPPARACEAE), *Coccinia grandis* (L.) J. Voigt Syn. *C. indica* Wight & Arn. (CUCURBITACEAE), *Coleus amboinicus* Lour. (LAMIACEAE), *Crotalaria laburnifolia* L. (FABACEAE), *Kalanchoe tubiflora* (HARVEY) Raym. - Hamet (CRASSULACEAE), *Leucas aspera* (Willd.) Link. (LAMIACEAE), *Mirabilis jalapa* L. (NYCTAGINACEAE), *Piper betle* L. (PIPERACEAE), *Solanum trilobatum* L. (SOLANACEAE), *Tridax procumbens* L. (ASTERACEAE) and *Withania somnifera* (L.) Dunal. (SOLANACEAE).

MATERIAL AND METHODS

Fresh leaves were cut into fragments with one edge of lamina left untrimmed. These fragments were boiled in 5-10% aqueous cupric sulphate for 1-2 minutes. Then 4 ml. of Con. HCl. was added and again boiled for 1-2 minutes. During these treatments, mesophyll tissue disintegrates. Being in contact along the untrimmed margin, the epidermal layers open out like a book. The peelings were placed on the slide with the outer side facing cover slip.

OBSERVATION AND RESULTS

Epidermal cell shape, size, cell wall features, stomatal types, stomatal size, distribution, stomatal index and glandular hair and non-glandular hair distribution are the parameters studied. They are presented in Table 1.

DISCUSSION

The epidermal characters either alone or in combination have been used in the pharmacognostic studies of several leaf drugs. In the present investigation 14 plant species of 14 genera belonging to 12 families were taken up for the study. Habit-wise, all the plant species are small herbs growing in fallow-lands and discarded places. The analysis of shape of epidermal cells reveals that it is varied from circular to elongated through oval, polygonal, rhomboid and irregular. The cells of

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Table 1 : Epidermal features of abaxial surface of leaves.

S. No.	Binomial	Shape of Epidermal cell	Size of Epidermal cell	Cell Wall	Predominant Stomatal type	Epidermal cells / mm²	Stomatal No./mm²	Stomatal Index	Stomatal size		G.H. No./ mm²	N.G.H. No./ m m²
									(L)	(B)		
1.	*Aloe barbadensis*	Polygonal	Large	Thin, Straight	Anamocytic	360	28	7	47	31	-	-
2.	*Boerhavia diffusa*	Rhomboid	Medium	Thin, Sinuous	Anamocytic	482	74	13	30	25	16	-
3.	*Catharanthus roseus*	Elongated	Small	Thick, Sinuous	Anisocytic	991	309	25	25	20	-	36
4.	*Cleome gynandra*	Polygonal	Small	Thin, Wavy	Anisocytic	632	203	22	25	17	8	-
5.	*Coccinia grandis*	Irregular	Small	Thick, Sinuous	Anamocytic	807	133	14	26	19	-	-
6.	*Coleus amboinicus*	Polygonal	Very large	Thin, Wavy	Anisocytic	132	355	73	22	13	2	10
7.	*Crotalaria laburnifolia*	Rhomboid	Medium	Thick, Sinuous	Anisocytic	460	183	28	29	22	-	2
8.	*Kalanchoe tubiflora*	Polygonal	Very large	Thick, Straight	Anisocytic	132	21	15	34	19	-	-
9.	*Leucas aspera*	Circular	Medium	Thin, Wavy	Anamocytic	592	194	25	29	21	45	6
10.	*Mirabilis jalapa*	Rhomboid	Small	Thin, Sinuous	Anisocytic	728	121	14	29	19	8	3
11.	*Piper betle.*	Elongated	Small	Thick, Straight	Anamocytic	865	72	8	30	21	8	14
12.	*Solanum trilobatum*	Irregular	Very small	Thin, Wavy	Anisocytic	1789	236	12	20	15	-	-
13.	*Tridax procumbens*	Oval	Very large	Thin, Wavy	Anamocytic	105	291	79	39	25	2	1
14.	*Withania somnifera*	Irregular	Large	Thin, Wavy	Anamocytic	246	621	69	30	23	-	13

G.H. - Glandular hair, N.G.H. - Non-Glandular hair, L - Length, B - Breadth.

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Coleus amboinicus, Kalanchoe tubiflora and *Tridax procumbens* are very large and that of *Solanum trilobatum* are very small while other plant species show medium, large or small cell size. The cell wall of nine plant species are thin while that remaining are thick. The cell wall is straight or wavy or sinuous. Anamocytic and anisocytic types of stomata have been recorded equally each in seven species. Maximum number of epidermal cells/mm² is recorded in *Solanum tribolatum* (1789/mm²) and minimum in *Tridax procumbens* (105/mm²). Stomatal number/mm² is highly variable with just 21 stomata in *Kalanchoe tubiflora* and 621 in *Withania somnifera*. Stomatal Index gives the ratio of stomata number to the epidermal cell number. It is as small as 7 in *Aloe barbadensis* and as large as 79 in *Tridax procumbens*. Of the plant species studied, *Solanum trilobatum* possesses stomata which are very small (20µ X 15µ) and *Aloe barbademis* possesses very large stomata (47µ x 31µ). Glandular hairs are present in seven plant species and Non-glandular hairs in eight. In *Coleus amboinicus, Leucas aspera, Mirabilis jalapa* and *Piper betle* both types of haris are present. The maximum number of glandular haris and non-glandular haris are seen in *Leucas aspera* (45/mm²) and *Catharanthus roseus* (36/mm²) respectively. The epidermal study of medicinal plants have been carried out by many workers and their study has confirmed that the characters related to epidermal cells, stomata and hairs can be successfully used in the proper identification of medicinal plants. The present study also confirms the earlier conclusions.

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Studies on Some Ethnomedicinal Plants from Gaganbawda Taluka of Kolhapur District

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Gaganbawda is one of the taluka from Kolhapur district situated in Western Ghats region showing prominent hilly areas covered with semi-evergreen forests inhabited by the tribal and non-tribal rural populations. The tribal and non-tribal rural populations from this taluka use various wild plants to treat different ailments and disorders. The present study deals with the information regarding some plants of ethnomedicinal importance used by rural populations in this taluka.

INTRODUCTION

Gaganbawda is one of the westernmost taluka from Kolhapur district covered with semi-evergreen forests. The populations in the taluka are residing far away from the urban area. Due to lack of the medical facilities in the interior areas of the taluka, the rural populations are dependant on the herbal remedies for the treatment of various disorders and ailments.

MATERIAL AND METHODS

A survey of plants of ethnomedicinal importance from Gaganbawda taluka was carried out during 2004-2006. The local informers and the traditional medicine practitioners were interviewed during the study period. The information regarding the ethnomedicinally important plants was noted. After receiving the information the plants were actually observed in the field, collected and identified scientifically.

RESULTS AND DISCUSSION

About nine different villages from interior parts of taluka were visited and individual rural medicine practitioners and local informers were interviewed. The information about the ethnomedicinal plants was noted indicating their botanical names, family, plant part used and the mode of application. Thirty one plant species belonging to twenty six angiospermic families were noted during the study period.

The earlier studies regarding the vegetation of this taluka are floristic accounts. Detailed study regarding the vegetation of the taluka from ethnomedicine view point is therefore necessary.

Table 1 : Showing ethnomedicinal plants from Gaganbawda taluka of Kolhapur district.

Sr. No.	Botanical name	Family	Plant part used	Mode of application
1.	*Alstonia scholaris* (L.) R. Br.	Apocynaceae	Bark	Juice of bark is given with buttermilk in the treatment of urinary disorders Juice of bark is given in Jaundice.
2.	*Ricinus communis* L. Errand.	Euphorbiaceae	Leaves and Root	Fresh Juice of the leaves is given in the treatment of jaundice. Paste of roots is applied to the waist to get relief from pains.
3.	*Gnidia glauca* (Fresen) Gilg.	Thymeliaceae	Bark	Crushed bark is used in the treatment of toothache.
4.	*Careya arborea* Roxb.	Lecythidaceae	Bark	Juice of bark is applied to the fractured bones.
5.	*Holarrhena pubescens* (Buch-Ham.) Wall. ex. G. Don.	Apocynaceae	Root	The paste of root is given with buttermilk in the treatment of amoebic dysentery.
6.	*Syzigium cumini* (L.) Skeels.	Myrtaceae	Seeds	Powder of dried seeds is given in the treatment of diabetes.

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7.	*Ficus glomerata* Roxb.	Urticaceae	Latex	Latex is applied to the wounds.
8.	*Clerodendrum serratum* (L.) Moon.	Verbenaceae	Leaves	Leaves are eaten as vegetable at the begining of monsoon, vermicidal in nature.
9.	*Aegle marmelos* (L.) Corr.	Rutaceae	Fruits and leaves	Fresh leaves are given in the treatment of diabetes. Ripe fruits are given in the treatment of amoebic dysentery.
10.	*Gloriosa superba* L.	Liliaceae	Rhizome	Crushed rhizomes are applied to the wounds of cattle.
11.	*Amarphophalus paeoniifolius* (Dennst) Nicols. Var. campanulatus (Decne.) Sivadasan	Araceae	Flower & Corm	Powder of dried inflorescence is given with cow's milk in the treatment of hemorrhoids. Corms are eaten as a vegetable in hemorrhoids.
12.	*Tridax procumbens* L.	Asteraceae	Leaves	Fresh Juice of the leaves in applied to wounds.
13.	*Leucas stelligiera* Wall.ex. Benth.	Lamiaceae	Leaves	Powder of dried leaves + owa seeds with coconut oil is applied to skin in the treatment of skin diseases.
14.	*Helicterus isora* L.	Sterculiaceae	Fruits	Paste of fruits is given in treatment of colic in children.
15.	*Caryota urens* L.	Arecaceae	Fruits	Paste obtained by rubbing fruits in water is applied to forehead in the treatment of migrain.
16.	*Gymnema sylvestre* (Retz.) R. Br. & S.	Asclepiadaceae	Leaves	Powder of dried leaves is given in the treatment of diabetes.
17.	*Acyranthus aspera* L. Var. aspera	Amaranthaceae	Stem	Stem pieces are tied to the parts bite by scorpion.
18.	*Mangifera indica* L.	Anacardiaceae	Resinous sap	Resinous sap from petiole is used in the treatment of discharge of pus from the ear.
19.	*Ziziphus rotundifolia* Lam.	Rhamnaceae	Root	Paste of root in cow's milk is used in the treatment of white discharge in women.
20.	*Artocarpus heterophyllus* Lam.	Urticaceae	Leaves	Ash of leaves is given with honey in the treatment of discharge of pus from the ear (oral administration).
21.	*Piper nigrum* L.	Piperaceae	Leaves	Ghee is applied to the leaves, made warm and applied to the inflamed parts of body.
22.	*Solena amplexicaulis* (Lam.) Gandhi	Cucurbitaceae	Root	Paste of roots is applied to the minor bums. Roots are chewed in the treatment of mouth ulcers.
23.	*Vitex negundo* L. Var. incisa (Lam).	Verbenaceae	Leaves	Paste of leaves is made hot and applied to the inflamed parts of body in treatment of rheumatism and backache.
24.	*Justicia adhatoda* L.	Acanthaceae	Leaves	Decoction of leaves is given in the treatment of cough.
25.	*Bauhinia racemosa* Lamk.	Caesalpiniaceae	Bark	Ash of leaves with water is used as ear-drops in the treatment of discharge of pus from the ear.
26.	*Allophyllus cobbe* (L.)	Sapindaceae	Leaves	The paste of leaves is applied to the body to cure Herpes.
27.	*Semecarpus anacardium* L.f.	Anacardiaceae	Resinous sap from Fruits	Resinous sap obtained from heated fruits is applied to wounds.
28.	*Smilax zeylanica* L.	Smilacaceae	Leaves, Fruits	Powder of dried leaves, fruits is given in the treatment of urine stone.

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29.	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Fruits	Dried fruit powder is given in the treatment of stomach disorders in the morning, (empty stomach)
30.	*Bambusa vulgaris* Schrad.	Poaceae	Leaves	The leaf juice is given in the treatment of amlapitta half cup. (empty stomach).
31.	*Psidium guajava* L.	Myrtaceae	Leaves	The fresh leaves with cumin seeds are given in the treatment of dysentery. A decoction of young leaves is given in the treatment of jaundice.

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Ethnomedicinal Observations Among Forest Dwellers of the Daitari Hill Ranges in Orissa, India

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Studies on hill tribes dependence on forest for their livelihood security are less in Orissa. Ethnomedicinal observations in the state with rich diversity of medicinal plants are still meagre. Present study enumerates 22 plant species belonging to 17 families used in treatment of various diseases among the tribes of Daitari Hill ranges in Orissa.

Keywords : Daitari Hill Ranges, Traditional Medicine, Indigenous Communities.

INTRODUCTION

Orissa has rich diversity of medicinal plants. But the medicinal plant sector is less documented Researches on medicinal plant diversity in Orissa, will contribute a lot to the livelihood of the indigenous community living in different ecological habitat. It will also provide traditional medicines for common diseases. As part of a comprehensive study on biomass utilization pattern in village eco-complex, this paper deals with traditional uses of medicinal plants by the indigenous people living in Daitari range of hills of Orissa.

The study area, Daitari Hill ranges (21°5'–22°10' N and 85°45'–85°50' E) comes under Keonjhar and Jajpur district of Orissa and covers an area of about 340.82 hectares of land. The area is connected with Brahmanipal town at a distance of 12 km. The express highway to Paradeep port passes at a distance of about 3 km. The area falls within Deogan and Sukinda Ranges of Rebana Reserve Forest. The hill ranges are marked by very steep slopes. North of the hills are densely forested. The study area lies in the tropical region where the climate is characterized by a very summer (March-June) and a cool winter (November-February). The average maximum and minimum temperature during summer and winter period ranges from 40°C to 23°C and 32° to 15°C respectively. The average annual rain fall is 1475.3 mm. The forests in the study area are tropical dry deciduous type (Plate No-1). Mining and allied activities and other anthropogenic activities have converted sum of the Northern Tropical Dry Deciduous forests into Dry Deciduous Scrub. The population of the area is dominated by the Scheduled tribes (75%) followed by Scheduled cast and other communities.

MATERIAL AND METHODS

Five selected hamlets of Daitari Hill ranges along with their tribal group like *'Mundas', Majhis'* and *'Sabars'* and in all respects the medicinal plants uses by the tribal are research materials.

Methodical approach was carried out in five randomly selected hamlets of Baliparbat area in Daitari range of hills of Keonjhar and Jajpur districts of Orissa. More than 60% of the population depends upon indigenous herbal drugs for their primary healthcare. The experimental villages were selected randomly representing different tribal communities (Plate No-2). From each village the village headman and elderly persons were interviewed to study their dependence on plants for treatment of diseases they suffer from (Plate No-3). Structured questionnaires were prepared to facilitate data collection as most of the respondents were illiterate. The native health-practitioners are known as "Baidyas". Structural interviews were conducted using questionnaires supplemented by group discussion. Voucher specimens of medicinal plants collected from the area were numbered and deposited in Herbarium of the P.G. Department of Botany, Ravenshaw University, Cuttack. Specimens were identified with the help of local flora. The information on wild and semi-wild plants were also scientifically identified and verified with the help of authentic literature (Behera and Mishra, 2005; Das and Mishra, 1988; Girach and Brahmam; Mishra and Ahmed, 2001; Nayak, Behera and Mishra, 2004; Saxena and Dutta, 1975; Saxena, Brahmam and Dutta, 1981).

RESULTS AND DISCUSSION

22 Plant Specimens with nomenclature are arranged alphabetically followed by family, local name, locality of collection and field number in parenthesis.

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. *Abrus precatorious* L., Fabaceae, Kaincha, Champanagar (25)

About 20 mg dry leaf powder is given orally with cold water once daily for about 15 days to cure leucorrhoea.

Freshly prepared gently warmed leaf paste along with mustard oil is applied upon rheumatic pain for relieving.

About 10 mg juice extract from seeds is given orally twice daily for 2-3 days for abortion in early stage of conception.

2. *Achyranthes aspera*. L., Amaranthaceae, Apamarang Nuasah (70)

Stem juice is applied on the root of teeth to stop bleeding from the gum.

About 20 mg. of leaf juice is administered orally to the pregnant woman for easy delivery.

Leaves and inflorescence are boiled and the decoction given orally twice daily to cure fever.

Root or leaf paste is applied to cure boils in early stage.

3. *Acorus calamus*. L., Araceae, Bacha, Bandhasahi (55).

Half teaspoonful of powder prepared from dried rhizome is given orally twice daily to cure asthma and once in morning in empty stomach to increase appetite. Rhizome is chewed for the treatment of dysentery.

4. *Aegle marmelos* (L.) corr., Rutaceae, Bela, Champangar (32)

Two or three fresh leaves are chewed daily in empty stomach to cure severe gastritis and to reduce blood sugar.

One teaspoonful dried leaf powder of Baels and Neem reduce blood sugar.

Leaf paste is applied upon affected part to cure injuries.

5. *Bauhinia vahlii* (Wt. & Arn.) Benth., Caesalpiniaceae, Siali, Bandhasahi (88).

Seed decoction (10 ml) is given orally twice daily in empty stomach for the treatment of diabetes.

About 20 mg dried seed powder is taken orally along with water to cure diarrhoea.

Leaf paste is applied to cure pimples on the face.

6. *Bombax ceiba* L., Bombacaceae, Simili, Nuasahi (80)

Fresh roots are grinded with water and applied on boils for relieving pain.

Paste prepared from fresh root along with sugar candy is taken by females to restore fertility.

7. *Dendrocalamus strictus* Nees, Poaceae, Salia Baunsa, Hurlabahali (42)

Wounds and cuts are treated with powder prepared from leaves and outer layer of stem.

8. *Erythrina varigata* L., Fabaceae, Paladhua, Nuasahi (74).

Inhaling of well crushed leaves by nostrils relieves headache.

9. *Ficus bengalensis* L., Moraceae, Bara, Nuasahi (72).

20 ml juicy paste prepared from tender tips of prop root is taken in empty stomach once daily for 21 days treatment of nocturnal emission and restiores vigour and strength in nerves if taken along with goat milk.

10. *Gymnema sylvestre* (Retz.) R. Br., Asclepiadaceae, Gudamari Champanagar (28)

Seven fresh leaves are chewed daily in morning in empty stomach for seven days to reduce blood sugar.

11. *Hemidesmus indicus* (L.) Br., Periplocaceae, Anantamula, Bandhasahi (98)

About 10 mg of root paste is taken with coconut water in empty stomach for seven days to cure jaundice.

Leaf juice (10 ml) is taken thrice a day to cure fever.

About 5 mg of root powder is taken with luke warm water thrice daily for about 21 days to cure rheumatic pain.

12. *Justicia adhatoda* L., Acanthaceae, Basanga, Daitari Hill top (102).

Leaf decoction (about 2 ml) taken with honey twice daily in empty stomach for 7-10 days to cure acute cough and cold.

Powder made from leaf is taken with warm water once daily for 20 days to get relief from rheumatic pain.

13. *Madhuca longifolia* (Koen.) Mac Br., Sapotaceae, Mahula, Vatisahi (50)

Powder obtained from dried leaves is applied to cure burn injuries.

Flower decoction (20ml) is taken twice daily for about 2 weeks for treatment of asthma.

14. *Ocimum sanstum* L., Lamiaceae, Tulsi Nuasahi (78)

Leaf juice with honey is taken in empty stomach for treatment of cold and cough. 3 to 4 fresh leaves are chewed along with honey daily in the morning in empty stomach to prevent malaria.

About 10 ml leaf juice is taken in empty stomach for 15 days to check excess urination.

15. *Pongamia pinnata* L., Pierre. Fabaceae. Karanja, Nuasahi (79)

Tender twig is used as tooth brush to cure pyorrhoea.

Oil extracted from seed messaged opin body to cure skin disease.

16. *Rauvolfia serpentine*. L., Benth.ex.Kur Apocynaceae, Patalagaruda Bandhasahi (53).

About 10 ml of root paste is taken orally for treatment of snake bite.

Bark obtained from root is grinded with water and about 10 mg of this paste is taken with cold water to cure stomachache.

About 5 mg root paste is taken twice daily in empty stomach for a week for reducing high blood pressure.

17. *Saraca asoca* (Roxb.) De Wilde, Caesalpiniaceae, Ashoka, Champanagar (20).

Powder prepared from dried petals of flower is taken with cold water twice daily in empty stomach to cure dysentery.

20 ml. of bark decoction is taken in empty stomach twice daily for about a month to cure irregular menstruation and excessive bleeding.

18. *Smilax wightii* Dc., Liliaceae, Muturi, Nuasahi (75)

20 ml of root decoction is administered orally for 15 days to cure rheumatic pain.

Root paste is applied externally for a week to cure veneral disease.

Powder of dried root is applied on chronic ulcers.

19. *Streblus asper* Lour., Moraceae, Sahada, Nuasahi (76).

Regular brushing of teeth by tender twigs cure toothache.

Root paste is applied externally against dog-bite.

Leaf paste with castor oil is applied to cure eczema.

20. *Syzygium cuminii* (L). Skeels., Myrtaceae, Jamu Koli, Champanagar (15).

20 ml fruit juice is taken once daily in empty stomach to cure dysentery.

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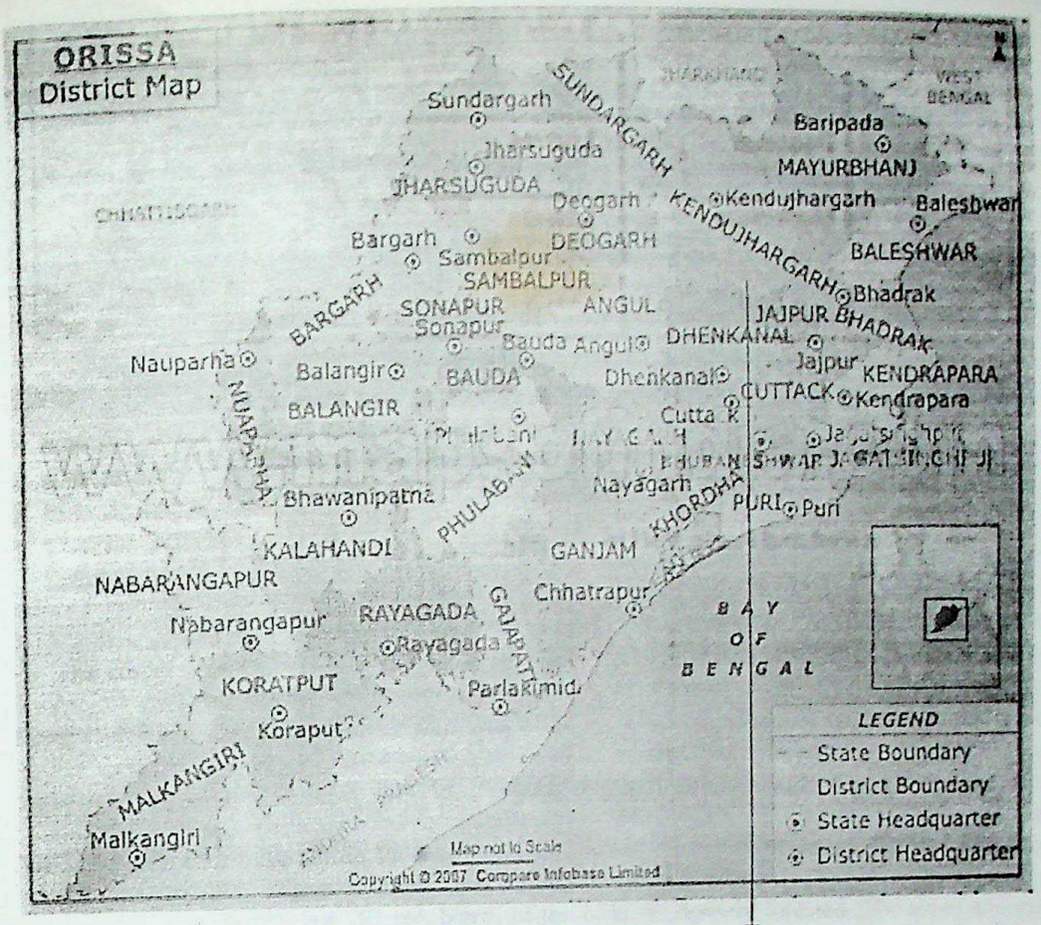

Study Area

10 mg of dry seed powder is taken in empty stomach for 7-10 days to reduce blood sugar.

21. *Terminalia bellirica* (Gaertn). Roxb., Combretaceae, Bahada, Bandhasahi (51).
    Powdered fruit is taken orally to get relief from indigestion and acidity.
    100 ml of decoction of fruit is taken orally once in early morning for 7 days for the treatment of piles.
22. *Thysanolaena maxima* (Roxb.), Kuntze, Poaceae, Phulajharu ghas, Daitarihill (105).
    Paste of the root is applied externally to get relief from pus producing wounds and cuts.

Floristic wealth of the tribal habitat provides various plant species which are used by the tribal people as food, fuel, fibre, house building materials etc. Besides these basic needs that are met with, the tribals of the study area heavily depend on the ecosystem for their primary healthcare. They have been using herbal medicine in a regular manner prepared from plant materials with strong religion-spiritual belief. The study reveals data on the uses of 22 plant species under 17 families for the treatment of different diseases. The herbal medicines are mostly administered in the form of juice, decoction, paste or powder, prepared in a crude method from different plant parts such as root, bark, leaves, flowers, fruits, seeds and whole plant. Tradition and beliefs are the only basis of use of these herbal medicines. The knowledge of this traditional healthcare system is passed generation after generation in oral form. The plant species identified as such for the treatment of different diseases, need chemical analysis to prove their efficacy.

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Short-Communication

Effect of Acid Scarification on Seed Germination and Seedling Growth in *Cuscuta reflexa* Roxb.

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Cuscuta, family Convolvulaceae, is a obligate angiospermic parasite. Majority of *Cuscuta* multiplies vegetatively through their stem. Its seed coat is hard, therefore, germination is very less (Lados, 1998 and Zaki *et al.*, 1998). Present work was taken upto to see the effect of chemical scarification on germination percentage and seedling emergence of *Cuscuta reflexa* Roxb. The seeds of *Cuscuta reflexa* were collected from different localities of Solapur District, Maharashtra and treated with concentrated sulphuric acid (H_2SO_4) for 30 minutes *i.e.,* chemical seed scarification. Scarified *Cuscuta reflexa* seeds were considered as treated and seeds soaked in distilled water for 30 minutes were considered as control. Treated and control seeds were kept, separately, in sterilized petri dishes (9 cm in diameter) on Whatman No. 1 filter paper moistened with 10 ml distilled water at room temperature (28 to 30ºC) and germination (%) was observed up to 120 hours at an interval of 24 hours. For seedling emergence studies chemically scarified *C. reflexa* seeds (treated) and control seeds were sown in an plastic tea pots at varying depths (0.5, 1, 1.5, 2 and 2.5 cm) @ 10 seeds/ pot at room temperature. The plastic pots filled with soil mixed with farm yard manure (FYM) in the proportion of 3 : 1 that was sterilized with 5 % formalin. Seedling emergence was recorded at 1 day interval up to 8 days after sowing and root shoot length measured.

Table 1 : Effect of seed scarification with concentrated sulphuric acid on germination percentage in *Cuscuta reflexa.*

Hours	Seed germination percentage	
	Control	Treated with conc. H_2SO_4
24	--	25
48	--	40
72	--	70
96	--	72
120	--	75

The first experiment was made to find the germination percentage of *Cuscuta* seeds. Due to very hard seed coat, *Cuscuta* seeds were unable to germinate in distilled water. So seed germination in distilled water was totally absent (Table 1). The hard seed coat of *Cuscuta* seeds were removed by concentrated sulphuric acid (H_2SO_4) treatment, chemical seed scarification resulted in seed germination upto 25, 40, 70, 72 and 75% after 24, 48, 72, 96 and 120 hours of sowing respectively (Table 1). Highest being after 120 hours of sowing. No germination was recorded in control.

In case of acid scarification treatment seedlings started emerging within 2 - 3 days from the 0.5 to 2.5 cm depth,

Table 2 : Effect of seed scarification with Conc. H_2SO_4 and sowing at different soil depths on seedling emergence and seedling growth in *Cuscuta reflexa.*

Seed sowing depth (cm.)	Seedling emergence (%)		Seedling growth (cm.)			
	Control	Treated	Control		Treated	
			Root	Shoot	Root	Soot
0.5	--	70	--	--	2.3	6.5
1.0	--	70	--	--	2.0	6.0
1.5	--	50	--	--	1.8	5.8
2.0	--	40	--	--	1.6	5.2
2.5	--	30	--	--	1.4	4.3

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however no emergene was recorded in control. Highest (70 %) emergence and seedling growth was observed in case of 0.5 and 1.0 cm seed sowing depth while lowest (30 %) emergence and seedling growth was recorded in case of 2.5 cm seed sowing depth (Table 2).

Results of present study find support from work of Mishra *et al*., (2003) who recorded that *Cuscuta* seeds required sulphuric acid treatment for breaking seed coat and dormancy and that maximum seedling emergence was recorded at the surface sowing, followed by 2 and 4 cm depth. Further increase in sowing depth reduced its emergence with no emergence beyond 8 cm depth.

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Short-Communication

Four Plant Species in the Category of 'Law of Signature' from Attapadi Hills, Western Ghats

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The early man gained the knowledge of curative power of plants based on many beliefs and assumptions. One of these is known as 'Law of Signature'. It was deducted from the apparent similarity of the plant and plant parts to the organs of human beings (Cotton, 1996). As such nature has provided us with plants for all diseases and has indicated them by plants and plants parts in the shape of the human organs for which they could be used. This perception guided the initial attempts of herbal remedies. Karatela *et al.*, (1991) reported use of the flowers of *Commelina benghalensis* which resemble the human eyes for ophthalmic treatment by Okpameri tribe in Akoko Edo of Bendel State of Nigeria.

The ethnobotanical survey conducted among the tribals of Attapadi hills in Western Ghats also revealed the use of four such plants. The women folk of Irulas of this area apply milky latex of *Cryptolepis buchanani* Roem. & Schult on their breasts after delivary to have their milk secretion increased. The twigs of *Mimosa pudica* L. (Touch-me-not) are kept tied to the cradle of babies to help them sleep quickly. The paste of roots of *Rauvolfia serpentina* (L.) Benth. ex. Kurz. (which look like snakes) is given internally to the patients bitten by snake. The bulbs of *Scilla hyacinthina* (Roth.) Macbr. (which look like boils) are baked in hot water and tied over the boils and wounds.

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Short-Communication

Effects of Pre-sowing Seed Treatment on Seed Germination in *Madhuca longifolia* L.

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Madhuca longifolia (L) is large evergreen tree well known for heart wood, flowers, fruits and seeds. The parts of plants are useful in preparation of medicine (Deokar 1998). Its seeds show hard seed coat dormancy, pre-sowing treatment of seeds with sulphuric acid, hot water, treatment have been shown to help breaking dormancy (Clemens *et al,* 1977, Pathak *et al,* 1980). It is therefore, present investigation taken up.

The experiment was conducted between December 2007 and February 2008 on seeds harvested between March 2007 and September 2007. Seeds were subjected to soak in water at room temperature served as control. Pre-sowing treatment with hot water and sulphuric acid as indicated in Table-1. After the treatment the seeds were placed in 100 ml of water for 16 hours for adequate inhibition followed by sterilization by slurry treatment with phenyl mercuric acetate. Seeds were kept for germination between moist filter paper in petridish at 20 ± 1ºC five replication of 25 seeds each pre-treatment were maintained.

Germination count were taken at regular interval (Table-1) seed, with 2 mm of exposed radical were regarded as germinated. The experiment was continued for 30 days, soaking seeds in water at 80ºC for 1 min. and at room temperature for 48 hours improved germination percentage on compared to control. Result of present study finds support from work of (Rolston 1978). Sulphuric acid treatment for 2 min. was effective in bringing about maximum germination in *Madhuca longifolia* L.

Table 1 : Effect of pre-sowing seed treatment with conc. Sulphuric acid and hot water on seed germination in *Madhuca longifolia* L. (Mean of five replicantes)

	Days after treatment and per cent germination							
Treatment	**2**	**8**	**12**	**16**	**20**	**24**	**26**	**30**
Control	00	2 ± 0.47	5	10 ± 0.97	13 ±	17	19.8	22
Soaking in water for 24 hrs at room temperature	01	05	31 ± 0.47	18	28 ± 1.24	34	37 ± 0.47	46 ± 0.97
Soaking in water for 48 hrs at room temperature	05	10	21 ± 0.49	40	47	50 ± 1.24	54	68
Hot water 80ºC for 1 min	00	11	22 ± 1.24	40 ± 0.94	49	52	55	72
Hot water 80ºC for 5 min	05 ± 0.47	06	22	19 ± 0.47	29 ± 1.24	35	38	52
Hot water 100ºC for 1 min	02	08	23	40 ± 0.47	50 ± 0.94	55	56	78 ± 0.94
Hot water 100ºC for 5 min	01	11	23	41 ± 0.47	50	51 ± 1.24	55	74 ± 1.24
Sulphuric acid for 1 min	02	10	22	41 ± 0.47	51 ± 1.24	52	56	75 ± 1.24
Sulphuric acid for 2 min	03 ± 0.94	12	25	45	55 ± 0.94	60	68 ± 1.24	79

± indicate standard derivation

Results of present investigation reveal that pre-sowing of seeds treatment with conc. Sulphuric acid for 2 min. was most effective in breaking hard seed coat dormancy followed by 1 min. hot water treatment at room temperature and 48 hours soaking treatment at room temperature. These results find support from work of Clemens (1977).

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Short-Communication

Allergenic Aerobiopollutants over Potato Fields in the District of Lakhimpur, Assam

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Aerobiopollutants in the form of fungi, bacteria and pollens of varieties of angiosperms as well as gymnospermic plants have in recent years attracted attention in relevance to human health. This is because, a large number of these propagules are responsible for a number of pathogenic human diseases. Bio-deterioration of materials in which the substrates and organisms interact, is usually a concern of air borne microbes (Gregory 1961). The analysis of composition, seasonal variation and the concentration of variety of bio-deteriorating microorganisms in the indoor, during packaging, storage and transit are of great concern these days (Minogue and Fry 1983).

The potato crop is affected by several fungal diseases and cause heavy losses to the crop either directly or indirectly. The presence of microbes in the air in the form of spores and mycelia has also been reported to influence the incidence of various diseases of crops. The present paper reports the fungal spore dispersal pattern in the environment over potato field and also comparison of spore concentration in cultivars of different degree of resistance to attack by pathogens.

The study was made in common potato fields plants with three different varieties of potato namely, *Khasi-garo* (partly resistant), *Asomiya alu* (susceptible) and *Kufri-jyoti* (resistant). PDA Petri plates of 9 cm diameter with 4 replications were exposed horizontally for 10 minutes between 4 PM to 5 PM at sampling site. Sampling were done twice during the month of October, 2007 (early) and January, 2008 (late), because potato crop is cultivated in the plains of Assam during the months from October to January. Exposed plates were incubated at 22 ± 2°C for 4 to 8 days. The colonies developed were examined and counted regularly. The colonies which could not sporulate after the treatment of induced sporulation were placed under 'sterile mycelium group'. The total number of colonies along with intensity of growth of different fungal colonies were recorded. The identification of different spore types was based mainly on comparative morphology and spore description and was subsequently confirmed by relevant literature (Ellis and Ellis 1985, Burnett and Hunter 1972, Tilak 1989). The spores which could not be identified due to their obscure nature or even otherwise, were placed under unidentified type. The identification of spore types was based on microscopic characters. Identification of fungal genera from PDA Petriplates was done on colony characteristics, growth and morphology.

Air is not a natural home for the microbes because they do not get necessary nutrients in this environment. The air borne microbes have a significant and vital impact on plant productivity, because, majority of air borne microbes cause diseases in plants (Chandrol and Karkum 2001). The spores or hyphae are carried by air, dew drops or rain water to the host plant surfaces where they establish and cause damage.

The data presented in Table 1 shows a total fourteen fungal microflora trapped from the fields during early and late stage of crop season. The identified genera belong to *Cladosporium, Penicillium, Rhizopus, Trichoderma, Chaetomium, Curvularia, Alternaria, Helminthosporium, Phytophthora, Aspergillus* and *Mucor* besides some obscure, sterile mycelia and unidentified organisms. The intensity and quantity of fungal microflora was maximum at the field of local cultivar with a total of as may as twelve types of fungi. Exposure of Petri plates in the field of cultivar *Khasi garo* revealed presence of about ten identified fungal propagules. The availability of *Trichoderma* and *Rhizoctonia* may also be a factor for less amount of microflora in the field planted with the cultivar 'Kufri Jyoti', because of reports of antagonistic effect of *Trichoderma* on few other microbes (Phukan and Baruah, 1990; Chandrol and Karkum, 2001). During the present investigation, the aerobiopollutants like *Cladosporium, Alternaria, Curvularia, Fusarium, Aspergillus* and *Mucor,* hyphal fragments and pollen grains have been reported to be responsible for inducing allergenic reactions to sensitive individuals (Agarwal and Shivpuri, 1974; Kulkarni and Karne, 2009). They may be significant potential source of allergens and inhalation of these biopollutants may be main causative factor for respiratory allergic diseases in human beings as reported by Shivpuri (1980) and Karne (2008). The role of ascospores of *Chaetomium* in causing allergy and acute asthma has been reported by Karne and Pandey (2006). The quantity of few fungal types such as *Cladosporium, Fusarium, Mucor, Alternaria, Cercospora, Chaetomium* and *Aspergillus* were higher during the early stage of potato crop in all the cultivars. This may be due to decreasing temperature and increased amount of humidity during the month of January resulting in the reduction of intensity of growth and sporulation of the microbes.

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Table 1 : The distribution of microbes in the air over three different potato cultivars of North Lakhimpur, Assam.

Sl.No.	Fungal Types	Number of colonies in Petriplates (Av. of 4 replicates)			Activity
		Khasi-garo	Asomiya alu	Kufri-jyoti	
1.	*Cladosporium* sp.	12.5	16.4	09.3	Allergenic
2.	*Penicillium* sp.	06.4	00.0	03.7	No clear
3.	*Rhizopus* sp.	07.6	08.2	03.4	Allergenic
4.	*Trichoderma* sp.	04.3	00.0	07.7	Antagonistic
5.	*Chaetomium* sp.	00.0	11.7	08.3	Allergenic
6.	*Curvularia* sp.	00.0	09.3	08.3	Allergenic
7.	*Alternaria* sp.	00.0	08.4	04.4	Plant pathogenic
8.	*Fusarium* sp.	07.4	07.6	07.2	Plant pathogenic
9.	*Cercospora* sp.	00.0	09.6	12.3	Allergenic
10.	*Aspergillus* sp.	08.2	08.9	03.6	Allergenic
11.	*Mucor* sp.	13.7	12.2	04.3	Allergenic
12.	*Phytophthora* sp.	02.4	08.2	04.3	Plant pathogenic
13.	*Rhizoctonia* sp.	00.0	00.0	00.0	Plant pathogenic Antagonistic
14.	*Helminthosporium* sp.	00.0	05.2	05.6	Plant pathogenic
15.	*Unidentified*	06.5	04.5	08.2	Not clear
16.	*Sterile mycelium*	04.7	05.6	02.4	Not clear

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Short-Communication

Ethnobotanicolinguistically Important Plants of Attapadi Hills, Western Ghats

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Attapadi hills, a part of undulating terrains of Western Ghats (10°46' - 10°59' N and 76°28' - 76°39'E) in Palghat district of Kerala State in bounded by Silent Valley Reserve Forests on West, Nilgiris on North, Anakatty on East and Aghali on South. It has a population of nearly 24000 in an area of 703.23 sq.km. settled in 129 hamlets (Kuttappam, 1996; Natarajan, 1997). Irulas, Madugas and Kurumbas are the three tribal groups inhabiting these hills (Menon, 1996). Their knowledge of local names and utility value of plants have been passed on from one generation to another verbally. It is observed that local names of few plants used by these tribals for different purposes are based on their utility value. A list of such plants is given in Table 1. Names of first seven plants are based on their medical properties, while that of the rest on other properties. The tribal healers also store an assortment of roots such as Thalavedhanaveru (=root curing head-ache) for the treatment of head-ache, Vayathukadiveru (=root curing stomach-ache) for the treatment of stomach-ache, Palluvedhanaveru (=root curing tooth-ache), for the treatment of tooth-ache, Sevitola (=leaf curing deafness) for the treatment of deafness and ear pain, and Sakaravyadhiveru (=root curing sugar disease) for the treatment of diabetes. However, their botanical identity could not be established as they keep the identity in a closely guarded secret.

Table 1 : Ethonobotanicolinguistically important plants.

Binomial and Family	Local name and its meaning	Therapeutic use/ethnobotanical value
Anaphyllum wightii Schott ARACEAE	Keerikizhanghu (=tuber consumed by mangoose)	Root is chewed by mangoose after an encounter with snake. Also used as antidote for snake bite by tribals.
Celastrus paniculatus Wild, CELASTRACEAE	Naakkupunnu veru (=root that cures the sores on the tongue)	Root is chewed for oral ulcer.
Cynoglossum zeylanicum Thunb. ex. lehm. BORAGINACEAE	Kaadhupaagha chedi (=herb that cures ear ache)	Extract of tuber is poured into ear to get relief from ear-ache.
Diplocyclos palmatus (L.) Jeffrey CUCURBITACEAE	Naayuni (=pests on dog)	Fruit pulp is a lice repellent for dogs.
Mallotus stenanthus Arg. EUPHORBIACEAE	Kathivetti (=knife injuries)	Leaf paste is applied on injuries, particularly of knife.
Pittosporum tetraspermum Wight & Arn. PITTOSPORACEAE	Analibegham (=medicine for the bite of viper)	Root is used for snake bite particularly viper.
Vernonia cinerea (L.) Less. ASTERACEAE albicans Dc.	Murivotti (= to Join the cuts)	Leaf paste is applied on cut wounds. Root paste is applied for bone fracture in cattle.
Crateva adansonii DC CAPPARACEAE	Mathalam (= drum)	Timber is suitable for making drums.
Cyperus rotundus L. CYPERACEAE	Paaykol (=grass used for weaving mats)	Grasses are used for weaving mats.
Grewia tillaefolia/Vahl. TILIACEAE	Sadachi (=plaited hair like structure)	Stem bark is used as rope.
Kydia calycina Roxb. MALVACEAE	Vellasadachi (=white plaited hair like structure)	Stem bark is used as rope.
Laportea crenulata Gaudich URTICACEAE	Aanaverati (=repulsive to elephant)	The plant, when comes in contact, is so much irritating that even elephants avoid them.
Lobelia nicotianifolia Roem. & Schult LOBELIACEAE	Kaatupugayila (=forest tobacco)	Leaves are rolled and used as tobacco.

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Short-Communication

Effect of Low Dose of Thidiazuron on Micropropagation of *Brassica alba*

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Thidiazuron, a substituted phenyl Urea (N-phenyl-N'-1, 2,3-thidiazol-5-ylurea; TDZ; Dropp), was reported to mimic the activity of cytokinin, as observed in the release of bud dormancy of apple (Wang *et al*, 1986), promotion of seed germination and also other activities like retardation of senescence and enhancement of nitrate reductase activity (Kerns and Meyer, 1985). Callus development was also induced for cytokinin dependent callus culture (Thomas and Kattermen 1986). Thidiazuron was reported to be an effective alternative of N^6-benzyl-cytokinin (Li *et al*, 2000; Mii *et al*, 2000).

Leaf discs of about 0.5 cm in diameter were excised from healthy mature plants of 60 days age and used as explants. Explants were washed thoroughly in detergent and then rinsed in distilled water. The explants were disinfected by rainsing in 0.1% mercuric chloride for 1 min followed by washing thoroughly in sterile water. The explants were then placed on callus induction medium containing basal MS salts (Murashige and Skoog 1962) fortified with 2 mg/l Benzylaminopurine (BA), 1 mg/l Kinetin(Kn), 3% sucrose and 0.8% agar. Cultures were maintained at 24 ± 2°C under 70% R.H. and at 16 hr photoperiod under illumination on 1200 Lux. Callii once induced were transferred in shooting media containing in addition to MS basal nutrients various doses of BA, Kn, Indole acetic acid (IAA), Zeatin (ZEA), Putresine (PUT), Spermidine (SPM) and TDZ. Cultures were maintained in triplicates and count of microshoot recorded after 21 days interval. In the pretreatment assessmentm, sets of two microshoots (formed on TDZ containing media) were taken and transferred in TDZ free media. Count of regenerated microshoots were recorded again after 21 days interval. Finally root development was noted in TDZ free rooting medium. Rooting medium contained half

Table 1 : Effect of TDZ on shootlet production. (Duration of culture :21 days ; values are mean of 15 observations)

Plant Material	Conc. of TDZ (M)	No. of shootlet
B. alba Leaf discs as explants	0 (Control)	3.5 ± 0.52
	1×10^{-7}	10.2 ± 0.90
	0.5×10^{-6}	9.5 ± 0.75
	1×10^{-6}	8.0 ± 0.56
	1×10^{-5}	7.10 ± 0.45
	1×10^{-4}	5.2 ± 0.89
	1×10^{-3}	3.8 ± 0.89
	L.S.D (5%)	1.985

Table 2 : Comparative account of different regulators for microshoot proliferation, (Duration of culture :21 days ; values are mean of 15 observations)

Plant Material	Treatment Conc. (M)	No. of shootlet
B. alba Leaf discs as explants	0 (Control)	3.5 ± 0.52
	BA 2×10^{-5}	7.5 ± 0.52
	Kn 2×10^{-5}	8.2 ± 0.56
	ZEA 2×10^{-4}	7.0 ± 0.86
	IAA 1×10^{-6}	6.5 ± 0.45
	PUT 1×10^{-5}	7.2 ± 0.78
	SPM 1×10^{-5}	7.2 ± 0.98
	L.S.D. (5%)	2.125

Table 3 : Effect of pretreatment with TDZ on shootlet proliferation. (After 21 days of culture values are mean of 15 observations)

Plant Material	Pretreatment Conc. (M)	No. of shootlet
B. alba Leaf discs as explants	0(Control)	3.5 ± 0.52
	1×10^{-7}	5.5 ± 0.75
	1×10^{-6}	7.2 ± 0.96
	1×10^{-5}	6.4 ± 0.89
	1×10^{-4}	4.2 ± 0.55
	L.S.D (5%)	1.595

Table 4 : Effect of pretreatment with TDZ on rooting. (After 21 days of culture values are mean of 15 observations)

Plant Material	Pretreatment Conc. (M)	No. of root
B. alba Leaf discs as explants	0(Control)	1.58 0 ± 0.51
	1×10^{-7}	3.6 ± 0.89
	1×10^{-6}	3.5 ± 0.78
	1×10^{-5}	0.8 ± 0.58
	1×10^{-4}	0.2 ± 0.98
	L.S.D (5%)	1.125

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strength of MS basal salts with 1 mg/l IAA. MS basal was used as control in case of microshoot development, but half strength MS basal was used as control for rooting.

TDZ facilitated in shootlet production in *B. alba* mainly in a dose lower than 10^{-6} M. 0.5 x 10^{-6} M and 1 x 10^{-7} M of TDZ induce better microshoot development than higher doses (Dhawan *et al* 2001) (Table 1.). Higher levels of TDZ (1 x 10^{-4} M and above) found to be inhibitory. Three cytokinins (BA, Kn, ZEA), one auxin (IAA) and two polyamines (PUT, SPM) were tested for comparison to TDZ with respect to facilitative microshoot proliferation. TDZ was found to be more effective in shootlet proliferation in much lower concentration such as maximum effect by TDZ was recorded in 1X10^{-7} M where as other growth regulators could induce comparable shootlet proliferation in a range 10^{-4} M to 10^{-5} (Table 2).

To study the lasting effect of TDZ, microshoots cultured in TDZ containing media were subcultured in TDZ free MS basal medium. After 21 days of culture, microshoots raised on medium containing 1 x 10^{-7} M of TDZ (supplemented in MS basal) were transferred in MS basal. Pretreatment in TDZ showed better microshoot proliferation as compared to control. (Table 3). For better root development higher doses of TDZ required. (Table 4).

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